

---

## The collection of blood and urine samples

(Originally created for project Euro-MOTOR - *SOP blood and urine FINAL 2011\_11\_28 netherlands.doc* in November 2011)

Name	Signature	Date
Prepared by		
Approved by		
Approved by		
Revised by		

## Introduction

The purpose of this SOP is to describe the instructions for the acquisition, processing, storage (and shipment) of blood and urine samples (as originally developed for the Euro-MOTOR project, which consists of questionnaires about environmental factors and sampling of urine and blood).

## Procedures

All incident patients are eligible for inclusion. Age, gender and geographically matched controls will be recruited if required.

Blood and urine should be collected as soon as possible after patients are diagnosed with Amyotrophic Lateral Sclerosis.

For controls blood and urine should be collected as soon as possible after inclusion in the a study.

## Required equipment at the time of collection

Portable Centrifuge (where necessary)

Cold pack for transport (4°C)

Plastic pipettes (x5 per subject)

Blood (per subject)

1x 10 ml silica vacutainers for serum (BD Cat No: 367820)

1x 10 ml lithium heparine vacutainers for plasma (BD Cat No: 367526)

2x 10 ml EDTA vacutainers for DNA isolation (BD Cat No: 367525)

1x PAXgene blood tube (BD Cat No: 762165)

Urine

10cc container for the collection of urine

Storage

2 ml cryovials for the storage the samples (x12 per subject) (Nalgene Cat. No. 5000-0020 / VWR Cat. No. 479-3222)

PAXgene Blood RNA kit (Cat nr: 762174, QIAGEN)

## Sample collection

Patients and controls should be matched and sample collection should be undertaken in an identical manner. It is very important to document the time of collection of the sample, the time of freezing, and the interval between collection and freezing. Intervals should be identical for patients in hospitals or at their own homes and for controls as far as possible.

Subjects should fast for at least 4 hours prior to the collection of blood and urine and the time of the last meal should be noted.

### *Serum samples*

- Collect blood in 1 silica vacutainer (red top). Invert gently for 6/7 times. Allow to clot for 1-2 hours on ice or 4 °C.
- Centrifuge the vacutainers at 1500g for 15 minutes.

- Aliquot the serum in 2ml cryovials, as 4×1ml aliquots. Label the cryovials for storage.
- Store the cryovials immediately at -80 °C.
- Always record exact time of collection (venipuncture), centrifugation and storage to -80 °C (on attached worksheet), and always record any deviation from the procedure.

### *Plasma samples*

- Collect blood in 1 lithium heparin vacutainer (green top). Invert gently for 6/7 times. Allow to clot for 1-2 hours on ice or 4 °C.
- Centrifuge the vacutainers at 1500g for 15 minutes.
- Aliquot the plasma in 2ml cryovials, as 4×1ml aliquots. Label the cryovials for storage.
- Store the cryovials immediately at -80 °C.
- Always record exact time of collection (venipuncture), centrifugation and storage to -80 °C (on attached worksheet), and always record any deviation from the procedure.

### *RNA samples*

- Collect blood in 1 PAXgene blood tube.
- Record the time of collection.
- Store samples at room temperature (approx. 18 °C) for a maximum of 24 hours.
- Store the samples at -20 °C before RNA extraction.
- Extract and store RNA using the PAXgene Kit according to the manufacturer's protocol.

### *DNA samples*

- Collect blood in 2 EDTA vacutainers (purple top).
- Record the time of collection.
- Store samples at room temperature (approx. 18 °C) for a maximum of 1 week.

### *Urine samples*

- Collect 10 ml of urine in a sterile collection tube with no preservative (e.g. Sterilin 128C). Leave 1-2 hours on ice or 4 °C.
- Centrifuge the urine sample at 1500g for 15 minutes.
- Aliquot the supernatant in 2ml cryovials, as 4×2ml aliquots. Label the cryovials for storage.
- Store the cryovials immediately at -80 °C.
- Always record exact time of urine collection, centrifugation and storage to -80 °C (on attached worksheet), and always record any deviation from the procedure.

ALL SAMPLES SHOULD BE CLEARLY LABELLED WITH A LOCAL CODE IN ORDER TO BE TRACEABLE.

## **Shipment of samples**

Samples should always be shipped together with the relevant sample data worksheet.

### *Plasma - Transport & packaging instructions*

To ensure safe transit, eppendorfs should be placed in appropriate containers as follows:

- Storage Box - BioBox 100
- Cardboard box with capacity for 100 tubes
- Ordered through VWR International - Product Number: 402/0510/00
- Samples should be shipped in suitable (insulated foam) containers, packed in sufficient dry ice to keep them frozen for the duration of the trip (2-3 days)

### *Plasma - Shipping compliance and labelling*

Samples shipped in dry ice require specific labelling and documentation to be completed. Please check with the courier for details, but as a minimum:

- The shipping container should be marked clearly with:
  - o the words 'Carbon Dioxide, 'solid' or 'Dry Ice'
  - o the words 'UN 1845'
  - o full name and address of the shipper and the consignee
  - o the net quantity of dry ice in the package
- A Class 9 label should be affixed to the container
- All irrelevant labels and marks should be removed
- Suggested courier service TNT or WorldCourier

ALWAYS give notice of shipment date to the receiving party before sending samples.

### *Serum - Transport & packaging instructions*

To ensure safe transit, eppendorfs should be placed in appropriate containers as follows:

- Storage Box - BioBox 100
- Cardboard box with capacity for 100 tubes
- Ordered through VWR International - Product Number: 402/0510/00
- Samples should be shipped in suitable (insulated foam) containers, packed in sufficient dry ice to keep them frozen for the duration of the trip (2-3 days)

### *Serum - Shipping compliance and labelling*

Samples shipped in dry ice require specific labelling and documentation to be completed. Please check with the courier for details, but as a minimum:

- The shipping container should be marked clearly with:
  - o the words 'Carbon Dioxide, 'solid' or 'Dry Ice'
  - o the words 'UN 1845'
  - o full name and address of the shipper and the consignee
  - o the net quantity of dry ice in the package
- A Class 9 label should be affixed to the container
- All irrelevant labels and marks should be removed
- Suggested courier service TNT or WorldCourier

ALWAYS give notice of shipment date to the receiving party before sending samples.

### *RNA - Transport & packaging instructions*

### *DNA - Transport & packaging instructions*

### *Urine samples - Transport & packaging instructions*

To ensure safe transit, eppendorfs should be placed in appropriate containers as follows:

- Storage Box - BioBox 100
- Cardboard box with capacity for 100 tubes
- Ordered through VWR International - Product Number: 402/0510/00
- Samples should be shipped in suitable (insulated foam) containers, packed in sufficient dry ice to keep them frozen for the duration of the trip (2-3 days)

---

### *Urine samples - Shipping compliance and labelling*

Samples shipped in dry ice require specific labelling and documentation to be completed. Please check with the courier for details, but as a minimum:

- The shipping container should be marked clearly with:
  - o the words 'Carbon Dioxide, 'solid' or 'Dry Ice'
  - o the words 'UN 1845'
  - o full name and address of the shipper and the consignee
  - o the net quantity of dry ice in the package
- A Class 9 label should be affixed to the container
- All irrelevant labels and marks should be removed
- Suggested courier service TNT or WorldCourier

ALWAYS give notice of shipment date to the receiving party before sending samples.