

## **EuroDSD WP05-1 Urine and Plasma Collection SOP**

**We would like to receive a urine** (ideally an aliquot of a 24-h urine collection) **and a plasma sample** collected **from each new patient entered into the EuroDSD database.**

We would like to have **samples both from patients with established diagnoses** (incl. CAH variants and AR insensitivity) **and patients who have no clear diagnosis yet.**

We would like to get **repeat samples from each patient** to study the age-dependent course of steroid production. Also, some disorders require confirmation with a follow-up urine if the first sample is a very early neonatal one.

Please send urine and plasma samples at the following time-points:

- **Neonatal** (or at diagnosis/first contact)
- **At the age of 1-2 months**
- **At the age of 12 months**
- **In annual intervals thereafter**

Please clearly complete the **age info** on the Sample Info Sheet WP05-2 for each sample you send.

To make it easy for you, we are providing three documents that are downloadable on the EuroDSD website:

- |                       |   |
|-----------------------|---|
| <b>EuroDSD WP05-1</b> | Overview and standard operating procedures  |
| <b>EuroDSD WP05-2</b> | Patient endocrine and sample sheet (needs to be completed for each sample sent)   |
| <b>EuroDSD WP05-3</b> | 24-h urine collection instruction and collection sheets (for parents, anonymised copies need to be sent to us with each urine sample) |



**EURO DSD Standard Operating Procedure (SOP) for 24-h urine  
collection, storage and shipment for subsequent gas  
chromatography/mass spectrometry (GC/MS) analysis (WP05)**

**Collection and storage of 24-h urine samples**

The urine collections will only be diagnostically meaningful if patients do not take interfering drugs, e.g. testosterone replacement, hydrocortisone replacement.

We are interested in samples from all patients with 46,XY DSD and 46,XX DSD.

Urine collections should be performed at initial diagnosis or first contact with the patient. If it is an early neonatal sample we will definitely need a follow-up sample at age 1-2 months.

Ideally we would like samples at neonatal age, at 1-2 months, at 12 months of age and thereafter in annual intervals.

**24-h urine in collection bottles**

24-h urine collections should be performed using plain collection bottles without any addition of preservatives.

Preferably the collection should be returned to hospital on the day of completion of the 24-h urine collection. If this is not possible, it is acceptable that the patient stores the bottle for up to 3 days at 4° Celsius (in the fridge) prior to transport of the bottle to the hospital.

Upon return of the collection bottle, the 24-h collection volume MUST be recorded. This information is crucial for the quantification of the steroid metabolite excretion.

The collection bottle should be gently converted several times and then two 10-ml aliquots should be taken e.g. in Sarstedt Urine Monovette® containers (10 ml). One sample should be stored locally at -20° Celsius and the other one should be sent to Birmingham.

Each sample should be labelled with the unique patient identifier, the date of sample collection and the 24-h collection volume in ml.

**24-h urine nappy collection**

Collections can only be carried out with nappies that contain fluid-absorbing granules such as Pampers, Huggies, Boots or similar. Collections CANNOT be EuroDSD WP05-1 v2 (Sept 2009)

carried out with eco nappies or cotton nappies. The same type of nappy should be applied throughout the collection day and parents should avoid putting creams on the baby's bottom.

The parents should provide information on the brand name and the size (age group) of the nappies. They should provide a clean nappy of the same size and brand as used for the actual collection. This Information will be used to determine the dry weight of the specific nappy.

In principle, we CANNOT make use of nappies that are contaminated with faeces ("poo") but only can process nappies that contain urine ("wee") only.

If upon changing the nappy only contains wee, the nappy can be put in the collection bag. If the nappy contains poo, it should be discarded. Odd stains and spills of poo do not represent a problem but a completely soiled nappy CANNOT be used.

If children pass a lot of poo or have diarrhoea the nappy collection should be postponed to 1 to 2 weeks later.

Parents should keep nappies in the -20 C freezer in a plastic bag that has been labelled with the name and date of collection.

In the clinical centre, the plastic bag with the collected nappies should be relabelled with the unique patient identifier, the date of nappy collection, the number of nappies in the bag and the time period covered by the collection. The nappy bag should be sent to Birmingham, directly transferred from the freezer into a styrofoam box by overnight courier express and include a dry, clean nappy we need for comparison.

### **Shipment of urine samples**

The 10-ml urine aliquots should be sent on dry ice; labelled plastic bags with nappies should be stored in a container sufficient to send them overnight express...) and the plastic bags with the nappies should be sent to Birmingham on dry ice via courier service, either immediately after aliquotting or after preceding storage of the sample at -20° Celsius.

Samples should be accompanied by a copy of the general sheet, the sample sheet and the anonymized copy of the urine collection sheet that has been completed by the parents (please delete name and day and month of birth for data protection purposes).

### **Samples should be shipped to:**

Prof Wiebke Arlt, School of Clinical and Experimental Medicine, University of Birmingham, Institute of Biomedical Research, Rm 225, Wolfson Drive Birmingham, B15 2TT, United Kingdom Email: w.arlt@bham.ac.uk

## **EURO DSD Standard Operating Procedure (SOP) for plasma collection, storage and shipment for subsequent liquid chromatography/ tandem mass spectrometry (LC/MSMS) analysis (WP05)**

**We are interested in samples from all patients with 46,XY DSD and 46,XX DSD.**

**Plasma or serum samples already available and stored at -20°C from historic visits are highly appreciated!**

**The plasma collections will only be diagnostically meaningful if patients do not take interfering drugs, e.g. testosterone replacement, hydrocortisone replacement.**

**Plasma collections should be performed at initial diagnosis or first contact with the patient. Neonatal patients should perform plasma collections on days 3-5, 30, 90 and thereafter at 6 months and 12 months of life, followed by annual collections.**

**Older individuals should provide annual plasma collections.**

**Further on we would like to measure samples from HCG or ACTH tests whenever performed for routine analysis. Standard recommendations for HCG and ACTH tests are given in the following. However other protocols will be welcomed.**

### **Collection and storage of plasma samples**

Plasma collections should be performed between 6 and 10 a.m. using heparin plasma containers (e.g. Li-Hep plasma). From historic visits / patients we can also accept serum samples. The minimum plasma volume for LC/MSMS analysis is 200 µl plasma, which can be processed from approx. **500 µl** blood. Larger volumes are welcomed. The plasma sample should be spinned down within 2 hours and the plasma should be transferred to a kryo tube resistant to dry ice (-78°C). The plasma samples should be stored locally at -20°C or colder. Avoid repeated freeze - thaw cycles!

Each sample should be labelled with the unique patient identifier and the date and time of sample collection. Please label also test substance and time point of the sample in regard to the start of the test if applicable.

## Shipment of plasma samples

The plasma aliquots should be sent to Kiel on dry ice via courier service, either immediately after aliquotting or after preceding storage of the sample at -20° Celsius. Samples should be shipped to:

PD Dr. med. Felix Riepe  
Universitätsklinikum Schleswig-Holstein  
Klinik für Allgemeine Pädiatrie  
Schwanenweg 20  
24105 Kiel  
Germany  
Tel +49-431-597-1797 or 1690  
Fax +49-431-597-1831  
Email [friepe@pediatrics.uni-kiel.de](mailto:friepe@pediatrics.uni-kiel.de)

## Standard procedures for stimulation testing

### HCG test

Initial blood sample taken between 6 and 10 a.m.  
Intramuscular injection of **5000** IE HCG/m<sup>2</sup> body surface.  
Second blood sample drawn **72h** (3 days) after HCG administration

### ACTH test

Initial blood sample taken between 6 and 10 a.m.  
Intravenous administration of 250 µg ACTH (Synacthen)  
(infants below 12 months of age 125 µg ACTH)  
Second blood sample drawn 60 min after ACTH administration

If the diagnosis in your institution is relying on other test protocols we will be happy to process the samples obtained with your standard test procedures. Please identify your test procedure on an accompanying letter. Otherwise we would prefer to use the SOP mentioned above.