SOP COLLECTION OF URINE

1.1 Urine collection

- Urine should be collected in a routine urine collection cup free of any preservatives and containing a lid. The cup should be appropriately coded according to own routine procedures.
- Direct the patient to provide first catch urine (approximately 50 ml of the initial urine stream) in the labeled urine collection cup (U00). This must be the first voided urine specimen. It is not allowed to use a catheter to collect the urine. ***Note****: If the patient can not stop his urine flow and provides more than the 50 ml, the entire volume will be kept for processing. If the subject is unable to provide this quantity, collect at least 24 ml.*
- Record the volume collected on the Urine Worksheet (Attachment 1).
- The urine collection cup must be maintained at 2-8°C in a fridge or cool box and processed **within 4 hours** of collection. Samples kept in a cool box should never come into direct contact with the ice pack, as this will damage them. Do not freeze unprocessed urine specimens.
- Record the following on the Urine Worksheet (Attachment 1):
    - ID
    - Year of birth
    - Barcode label
    - Date and time of collection
    - Amount of collected urine
    - Consumption of water prior to voiding

1.2 Processing, aliquoting and storage of urine specimens

The urine needs to be processed within 4 hours after collection. The urine processing starts with the preparation of full urine specimens (section 1.2.1).

1.2.1 Preparation of full urine specimen (Sample U04, U05)

Note: *In case less than 50 ml urine is collected, check the priority scheme to adjust the number of cryovials.*

- Wear protective clothing, gloves and eye/face protection when handling the NG urine transport medium tubes. Note: the NG urine transport medium tubes can be stored at room temperature.

  Invert the urine collection cup five times to resuspend the cells. Transfer about 8 ml of urine to the labeled MDx urine preservation vial untill it is filled between the minimum and maximum urine fill lines as indicated on the tube (1x U05). This should be done immediately after collecting the urine.
- Transfer 4 ml of urine to the labeled Full urine cryovials (2-3x U04). Put the urine collection cup back at 2-8°C in a fridge or cool box.
- Screw on the cap tightly and place the U05 and 2-3 U04 vials immediately at -80°C or lower in a labeled ratiolab® Cryo Box.
- Record time of transfer of urine into the MDx Preservation vial, time of freezing and the ID of the box in which the sample is stored on the Urine Worksheet (Attachment 1)
- Proceed with section 1.2.2.

1.2.2 Preparation of urine supernatant (Samples U01)

- Centrifugal tubes used for the preparation of urine supernatant should be appropriately coded with the ID according to own routine procedures.
- Transfer 30 ml of urine from the urine collection cup in a 50 ml conical centrifuge tube labeled with the ID (U01).

  Note: *In case less than 50 ml urine is collected, check the priority scheme to adjust the volumes and number of cryovials.*
- Centrifuge the U01 tube at 4°C for 20 minutes at 500 g. The brakes of the centrifuge are switched **OFF** *(Stopping without brake will take about 5 minutes).*
- Label 3-7 Sarstedt cryovials with a barcode according to section 1.3 (U01a-g).
- Pipet 4 ml of the supernatant from the U01 tube into each of the labeled cryovials.
- Store the 3-7 cryovials with urine supernatant immediately at -80°C or lower in a labeled Sarstedt Cryobox.
- Record time of centrifugation, time of freezing and the ID of the box in which the samples are stored on the Urine Worksheet (Attachment 1).
- Proceed with section 1.2.3 for further processing of the remaining material.

1.2.3 Urinary Sediment (Samples U02, U03)

- Centrifugal tubes used for the preparation of urine sediment should be appropriately coded with the ID according to own routine procedures.
- Mix the sediment in the U01 tubes obtained at 1.2.2 with 2 ml ice-cold buffered sodium-chloride solution pH 7.4 (PBS) and transfer the resuspended sediment to two Sarstedt cryovials labeled with the ID (U02 and U03).
- Centrifuge the U02 and U03 cryovials at 4°C for 10 minutes at 3000 g. The brakes of the centrifuge are switched **ON**
- Discard the supernatant from each cryovial.
- Immediately freeze the two cryovials with urine sediment in liquid nitrogen and subsequently place it into a freezer unit of -80°C or lower in a labeled Sarstedt Cryobox. If freezing in liquid nitrogen is not feasible, then the cryovials should be immediately frozen at -70°C or lower. The method used for freezing should be recorded on the Urine Worksheet (Attachment 1).
- Record the ID of the box in which the samples are stored on the Urine Worksheet (Attachment 1).

Notes:

- *The staff who processes the samples is responsible for:*
  i. ensuring that the samples are processed, labeled, documented and stored as per this protocol.
  ii. ensuring that the health and safety guidelines pertaining to that particular workplace are adhered to.
- *The freezer used for storage needs to be in a well-ventilated or air-conditioned room.*
- *Power needs to be provided by the "emergency back-up" electrical supply in case of a power cut.*
- *The freezer should be controlled by an alarm system.*

1.3 Summary of information to be recorded on the Urine Worksheet

The following information should be recorded on the Urine Worksheet (Attachment 1):

- ID
- Year of birth
- Barcode label
- Date and time of collection

- Amount of collected urine
- Consumption of water prior to voiding
- Tick off samples processed
- Start time of urine transfer to tubes for samples U04a-c and U05
- Start time of centrifugation of samples U01
- Date and time of freezing of samples U04a-c and U05
- Date and time of freezing of samples U01a-g, U02 and U03
- Method of freezing of samples U02 and U03
- Name of person who processed the samples
- Storage box ID

Attachment 1

URINE WORKSHEET GROUP 2

Study ID: _ _ *. _ _ _ _ **Date of collection _ _ - _ _ - _ _ _ _**

Year of birth _ _ _ _ **Time of collection _ _. _ _ hours**

Amount of collected urine ___ ml

Barcode

Tick if processed	**Fill in the number of Cryovials**	**cap**	**ID storage box**
□ **sample U01a-g:** Urine supernatant	_ _	black cap	_ _ _ _ _ _ _ _ _ _
□ **sample U02:** Urine sediment	**1**	brown cap	_ _ _ _ _ _ _ _ _ _
□ **sample U03:** Urine sediment	**1**	brown cap	_ _ _ _ _ _ _ _ _ _
□ **sample U04a-c:** Full urine	_ _	white cap	_ _ _ _ _ _ _ _ _ _
□ **sample U05:** Full urine (MDx tube)	**1**		_ _ _ _ _ _ _ _ _ _

Start time of urine transfer into tube for U05: _ _. _ _ hours

Storage date and time in freezer samples U05: _ _ - _ _ - _ _ _ _ / _ _. _ _ hours

Start time of centrifugation sample U01: _ _. _ _ hours

Storage date and time in freezer samples U01a-g: _ _ - _ _ - _ _ _ _ / _ _. _ _ hours

Storage date and time in freezer samples U02 and U03: _ _ - _ _ - _ _ _ _ / _ _. _ _ hours

Samples processed by (name): _____

Attachment 2

Ordering information

Product	***Supplier***	***Article no.***	***Description***
Sarstedt Micro tubes 2.0 ml, Type I with skirted base	Sarstedt	72.609	Cryostorage tubes and urine sediment U03, U04
Sarstedt colour-coded cap Brown	Sarstedt	65.716.009	Urine sediment U02, U03 (wsch in 5ml cryovials)
6 ml cryovials	Micronics	MP32301	Urine supernatant U01a-g, Full urine U04a-c
screw caps 6 ml tube – black	Micronics	MP53222	Urine supernatant U01a-g,
screw caps 6 ml tube – white	Micronics	MP53201	Full urine U04a-c
MDx Urine preservation tube	MDx Health	Supplied by Erasmus MC	Full urine U05
Conical centrifuge tube 50 ml	Corning Life Sciences	430829	Tubes for processing urine supernatant