ReBioLup

STANDARD OPERATING PROCEDURES

SAFETY CONSIDERATIONS

Please use gloves when handling blood and urine samples. Perform collection and partition of samples in a place designated for work with blood and urine.

LABELLING OF THE SAMPLES

Make sure that all material is labelled according to the following algorithm:

REBIOLUP-XXX-YYY-ZZZ

where:

XXX designates centrally provided patient's randomisation number,

YYY designates the visit (BSL, M12, or M24),

ZZZ designates the nature of the specimen (SRM for serum, URN for urine, GEN for PAXgene tube, KID for kidney biopsy slides and ELM for kidney tissue fragments intended for electron microscopy).

BLOOD SAMPLING (FOR SERUM AND PAXGENE)

Material

Serum tubes with yellow lid and gel (e.g., 5 mL Becton Dickinson AB 366566); PAXgene Blood RNA tubes; 0.5 mL microtubes (e.g., Sarstedt AB 72.730.005); transfer pipettes and tips.

Method

Blood for serum must be drawn before the blood to PAXgene Blood RNA tube. Butterfly needle is preferable for the blood sampling. The result will be:

2 x 5 mL serum tubes, to yield 16 x 225 µL serum in microtubes

1 x 2.5 mL PAXgene Blood RNA tube

Blood-filled tubes without anticoagulant will be centrifuged (2,000 x g for 10 minutes) from 30 minutes (minimum) to 180 minutes (maximum) after blood collection, with maximum acceleration and deceleration at 22 °C. The supernatant (serum) will be transferred into 16 labelled (as described above) 0.5 mL microtubes containing 225 µL serum/tube. Close the microtubes carefully with the provided lids and place them immediately in vertical position in a freezer (-20°C or below, preferably -80°C). The samples may be stored temporarily at 4°C for a short period of time (≤6 hours) pending transfer to the freezer. Transfer the frozen samples within no later than 72 hours (preferably immediately) to a -80˚C freezer.

Blood-filled PAXgene Blood RNA tubes will be labelled as described above and be kept at room temperature for 2–72 hours before being frozen at -20°C. Later, they should be moved to a -80°C freezer within 2 months.

URINE SAMPLING

Material

50 mL tubes (e.g., Sarstedt AB 62.547.254); 2 mL microtubes (e.g., Sarstedt AB 72.694.005); transfer pipettes and tips.

Method

1. The urine sample should be obtained from one of the first morning voids in a plastic cup and transferred directly into a 50 mL tube.
2. Store the urine in 4 °C until centrifugation.
3. Centrifuge the urine at 2,500 x g for 10 minutes, no later than 60 minutes after the urine capture.
4. Fill 10 microtubes, each with 1 mL of the supernatant from pelleted urine.
5. Close the tubes carefully with the provided lids and place them immediately in vertical position in a freezer (-20°C or below, preferably -80°C). The samples may be stored temporarily at 4°C for a short period of time (≤6 hours) pending transfer to the freezer. The frozen samples should be then transferred to a -80˚C freezer within no later than 72 hours (preferably immediately).

PROCESSING OF THE KIDNEY BIOPSIES (FOR LIGHT AND ELECTRON MICROSCOPY)

Two very small fragments of the kidney cortex will be isolated from the freshly obtained biopsy core before any other procedure in order to perform electron microscopy studies. Each one of these two fragments should ideally contain at least one glomerulus. These two fragments, no larger than a pinhead, must immediately be dropped into a 2.5% glutaraldehyde solution (together). Samples will then be stored at 4°C until shipment to the coordinating centre.

When the material for electron microscopy is obtained, the biopsies will be processed as per local practice to perform immunofluorescence analyses (on frozen material) and light microscopy analyses (on slides obtained from the paraffin-embedded block).

RENAL PATHOLOGY SLIDES

At a certain stage of the study, investigators will be asked to provide unstained slides from paraffin-embedded blocks of residual renal tissue, which meanwhile have been stored in local biobanks. These slides are intended for research purposes.

In brief, 15 unstained slides (4 µm thick) will be obtained (microtome) from the paraffin-embedded blocks. These additional research slides should not be prepared until so requested, in order to avoid degradation of the tissue over time and in varying conditions (*e.g.*, tissue aging). The slides should be labelled as described above.