Blood sampling manual (T0 – T3 – T6)

Samples to be drawn:

- 1x Citrate 3 ml
- 1x EDTA 6 ml
- 1x EDTA 3 ml *(to be kept on ice directly after sampling)*

} For plasma storage

- **Additional** tubes for **standard laboratory** measurements
  *(number / type of additional tubes depending on local practice, see **appendix B**)*

NB Please process all blood samples **within 2 hours after sampling**.

Sampling manual:

1. After blood drawing, turn tubes several times to mix with additives. Do not shake! NB the Citrate tube needs to be completely filled!!

2. Process the tubes as follows;
   - Put the EDTA 3 ml tube immediately after sampling (within 5 minutes) **on ice.** (This tube does not need to be centrifuged!).
   - **Centrifuge** the Citrate 3ml and the EDTA sample 6 ml according to lab centrifuge instructions in appendix A.
   - Send the additional tubes for standard lab to the laboratory (labelled with Name, patient hospital ID etc.). See appendix B for required measurements.

3. **Label** all cryovial containers (10 in total) as follows:

   NAC **-** [Randomis. No.] **-** Time point T0 / T3 / T6 **-** EDTA / Citr / Whole blood

   *Example: NAC **-** 511020 - T3 - Citr* or *NAC - 610001 - T0 - Whole blood*

4. Transfer ≥500 µl of the EDTA 3 ml tube on ice (whole blood) to a cryovial container (make sure to mix the blood again before transferring) and close with (red) cap.
   NB Keep on ice until storage in freezer!

5. Transfer all vials, including centrifuged plasma, to a cryovial box and store in **-80°C freezer**.

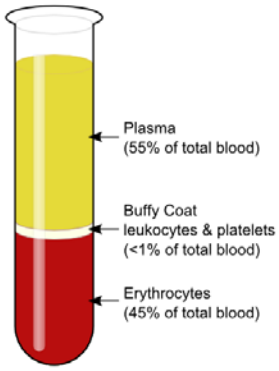
   With sufficient material, the following **10** cryovials should be ready for storage;
   - 1x ≥500µl whole blood (on ice) – red cap
   - 6x ≥500µl EDTA plasma – purple cap
   - 3x ≥500µl Citrate plasma – blue cap

Appendix A: Lab centrifuge manual for plasma storage

Processing manual for venous blood

Samples to be processed:

- 1x Citrate 3 ml
- 1x EDTA 6 ml



1. Centrifuge Citrate 3ml tube at room temperature (18˚C), **20 minutes at 1600 RCF**.
   a. Transfer supernatant plasma carefully to a new tube (remove plasma until ± 0.3 cm above the buffy coat to prevent interferences)
   b. Pellet and old tube can be disposed of.

2. Centrifuge the supernatant citrate plasma tube ánd the EDTA 6ml tube at room temperature (18˚C), **15 minutes at 2800 RCF**.
   During centrifugation, all cryovials can be labelled (see point 3 sampling manual).

   a. Divide the EDTA plasma over **6** cryovial containers (with at least 500 µl per container) and put (purple) cap on.

   b. Transfer the **upper ¾ part** of the citrate plasma to **3** cryovial containers (with at least 500 µl per container) and put (blue) cap on.

3. Check if all cryovial containers are labelled correctly and prepare for storage in freezer (see point 5 sampling manual).

4. All remaining containers and cell pellets can be disposed of.

Appendix B: Required standard measurements through regular lab

	Chemistry	Hematology	Endocrinology
Only at baseline (T0)		- Hb-typing *(Either electrophoresis or HPLC)*	- *β-HCG* *(NB Only required in women in fertile age, in blood or urine)*
All visits (T0, T3, T6)	- Bilirubin (total) - C-Reactive Protein (CRP) - Creatinine - Lactate Dehydrogenase (LDH)	- Hemoglobin - Erythrocytes - MCV - Reticulocytes - Leucocytes - Trombocytes	