TARDIS - Standard Operating Procedure, No. 029

Title: Collection & storage of blood samples.

1. TARDIS blood samples are collected at baseline, day 7 and day 35. All samples are mandatory for every patient unless under exceptional circumstances, as they are all part of the main trial. The DNA sample can be omitted if the patient refuses this and a separate DNA consent form has not been signed by the participant.

2. After a patient has been consented into the trial, up to 5 samples of blood can be collected:

a.	1 x Full Blood Count	(Baseline, Day 7 and Day 35)
b.	1 x 4mls EDTA. (DNA)	(Baseline OR Day 7 OR Day 35)
c.	1 x 4mls EDTA. (plasma)	(Baseline, Day 7)
d.	1 x 8mls clotted sample (serum)	(Baseline, Day 7)
e.	1 x P-Selectin	(Baseline, Day 7)

a. FULL BLOOD COUNT

Baseline sample: Results will be used at randomisation, from the sample taken for clinical use, after the onset of stroke/TIA and before randomisation.

Day 7 and Day 35 FBC: taken at time of face-to-face visit.

b. EDTA (DNA)

This is the DNA sample and is optional if the patient does not consent on a separate DNA consent form. This can be taken anytime up to and including the Day 35 visit. Only one sample per recruit is required.

The tube is frozen whole at less than or equal to -20 degrees centigrade in its collection bottle (preferably -80 degrees centigrade). Transport to Nottingham Co-ordinating Centre will be arranged during the course of the trial.

c. EDTA (plasma)

This sample is centrifuged to collect and freeze the plasma. Transport to Nottingham Co-ordinating Centre will be arranged during the course of the trial.

Note: If a centre does not use blood bottles containing EDTA, then their bottles usually used for FBC samples is sufficient (this will contain appropriate anticoagulant).

d. CLOTTED SAMPLE (serum)

This sample is centrifuged to collect and freeze the serum. Transport to Nottingham Co-ordinating Centre will be arranged during the course of the trial.

e. P-SELECTIN (special pack supplied to sites)

Collected via one special monovette tube and transferred into 3 smaller tubes (see separate working practice documents, 017 and 017a).

3. Spin c) and d) samples in a centrifuge at 3000rpm for 9 minutes, remembering to balance the tube/s. Pipette as much of the plasma/serum out as possible and transfer to separate 5ml sterile tubes (avoid contaminating this with blood). Please use transfer tubes large enough to label as below – 1 tube per participant, per sample. Please note that most eppendorf tubes are not of an adequate size.

4. Label each of the samples b), c), and d) by entering the following information, using a pen that is smudge proof and fade-free at ultra low temperatures:

   - Date taken
   - Full patient ID (Centre number, trial number, initials)
   - DNA, plasma or serum on the appropriate samples
   - Which visit it refers to i.e. baseline, Day 7, Day 35

5. Freeze b), c) and d) at –20 degrees or less, ideally at -60 to -80 degrees centigrade.

6. Enter the data on the trial blood sample freezer log form, located in the Investigator Site File and on our website (http://www.tardistrial.org/jevpybki.htm.

7. If you have storage issues, please contact the Nottingham Co-ordinating Centre to organise transportation to them.

8. The UK Co-ordinating Centre will arrange collections periodically. Prior to transportation to Nottingham you will be required to fax over the completed freezer logs so that the courier knows how many boxes/dry ice to supply for collection.

9. The cost will be borne by the UK Co-ordinating Centre.