SOP COLLECTION OF BLOOD

The following blood specimens are collected using vacutainers:

Sample no.	*Specimen*	*Blood volume (ml)*	*Becton Dickinson Tubes*
B01	Serum	8.5	Vacutainer SST II Advance
B02	Serum	8.5	Vacutainer SST II Advance
B03	Plasma (EDTA)	8.5	Vacutainer PPT K_2EDTA 15.8 mg
B04	Plasma (EDTA)	8.5	Vacutainer PPT K_2EDTA 15.8 mg
B05	RNA	2.5	PAXgene Blood RNA tube (containing 6.9 ml RNA stabilization additive)
B06	Whole blood (for DNA extraction)	3	Vacutainer EDTA 3 ml

1.1 Blood collection

- Ensure that the vacutainer tubes are appropriately coded according to own routine procedures.
- Collect the blood samples according to the institutional routine clinical procedures.
- Tick off the collected samples on the Blood Worksheet (Attachment 1)
- Record the time of collection on the Blood Worksheet (Attachment 1).

1.2 Processing of blood specimens

Processing of Serum tubes (samples B01, B02):

- Rack the tubes upright at room temperature for 30 minutes (minimum 30 minutes, maximum 60 minutes) to encourage clotting.
- Invert the blood tubes gently (10 to 15 times) and then place them in the centrifuge.
- Centrifuge the tubes at 2200 g (rcf) for 10 minutes (uncooled).
- If necessary, serum samples can be centrifuged for 20 minutes along with plasma samples from the next patient.
- Record the time of centrifugation on the Blood Worksheet (Attachment 1).
- After centrifuging the specimen tubes should be put up right into a rack in a fridge or cool box until aliquoting and freezing (see section 1.4). Samples should be handled carefully from this point on so as not to disturb the gel seal.

Processing of EDTA Plasma tubes (samples B03, B04):

- The plasma tubes should be processed within 4 hours of being taken
- Invert the tubes gently (10 to 15 times) and then place them in the centrifuge.
- Centrifuge the tubes for 20 minutes at 2200 g (rcf) (uncooled). If there are serum samples from the previous patient, these could be included in this centrifuge cycle.
- Record the time of centrifugation on the Blood Worksheet (Attachment 1).
- After centrifuging the specimen tubes should be put upright into a rack in a fridge or cool box until aliquoting and freezing (see section 1.4). Samples should be handled carefully from this point on so as not to disturb the gel seal.

Processing of RNA tube (sample B05):

- Keep upright at room temperature for 2 hours before freezing (see section 1.4).
  The PAXgene Blood RNA tube contains 6.9 ml of Proprietary RNA stabilization additive resulting in a total volume of 9.4 ml.
- Do not centrifuge the sample!

Processing of Whole Blood tube (for DNA extraction, sample B06):

- Keep in a cool box until freezing (see section 1.4).
- Do not centrifuge the sample!

Warning: Samples kept in a cool box should never come into direct contact with the ice pack, as this will damage them.

Note:

- *If samples cannot be processed within the time-frames indicated above, continue the processing and take care of exactly recording the requested information regarding processing times on the Blood Worksheets.*

1.3 Transport of processed blood specimens for aliquoting and storage

If the processed blood specimens are to be transported to another laboratory for further processing and storage (see section 1.4), please follow these instructions:

- As stated earlier, the samples need to be handled carefully so as not to disturb the gel seal.
- Keep the blood specimens upright in a rack.
- Transport the blood specimens in a cool box to the laboratory.

1.4 Aliquoting and storage of blood specimens

- Label the following number of Sarstedt cryovials with a barcode according to the instructions provided in section 1.3:
  - 2x 2 ml cryovials for each serum specimen (samples B01a-b, B02a-b)
  - 2x 2 ml cryovials for each plasma specimen (samples B03a-b, B04a-b)
- Pipet 1.5 ml of each serum or plasma specimen into the corresponding labeled cryovials.
- Use colour-coded Sarstedt caps to identify the different cryovials in the following way:

Samples	*Specimen*	*Total number of cryovials*	*Cap colour*
B01, B02	Serum	2 x 2 = 4 (2 ml cryovials)	Yellow
B03, B04	Plasma (EDTA)	2 x 2 = 4 (2 ml cryovials)	Violet

- Store the cryovials in a labeled Sarstedt Cryobox at -80°C or lower.
- Store the PAXgene RNA tube (B05) and the whole blood tube (B06) into corresponding cryoboxes.
- Record the time of freezing on the Blood Worksheet (Attachment 1).
- Record the ID of the boxes in which the specimens are stored on the Blood 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.*

1.5 Summary of information to be recorded on the Blood Worksheet

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

- ID
- Year of birth
- Barcode label
- Date and time of collection
- Name of collector
- Tick off collected specimens
- Name of person who processed the samples
- Time of centrifugation for specimens B01 to B04
- Start time of keeping sample B05 at room temperature for 2 hours
- Start time of keeping sample B06 upright in a coolbox
- Date and time of freezing of the specimens
- Storage box ID

Attachment 1

BLOOD WORKSHEET

Study ID: _ _ *. _ _ _ _

Year of birth _ _ _ _

Barcode

Date of collection _ _ - _ _ - _ _ _ _

Time of collection _ _. _ _ **hours**

Name of collector _____

Tick if collected		**Cryovials/cap**	**ID storagebox**
□	**sample B01a-b:** SST II advance (yellow) 8.5 ml	**2** yellow cap	_ _ _ _ _ _ _ _ _ _
□	**sample B02a-b:** SST II advance (yellow) 8.5 ml	**2** yellow cap	_ _ _ _ _ _ _ _ _ _
□	**sample B03a-b:** PPT K2E, 15.8 mg, 8.5 ml	**2** violet cap	_ _ _ _ _ _ _ _ _ _
□	**sample B04a-b:** PPT K2E, 15.8 mg, 8.5 ml	**2** violet cap	_ _ _ _ _ _ _ _ _ _
□	**sample B05:** PAXgene blood RNA 2.5 ml	**1** tube	_ _ _ _ _ _ _ _ _ _
□	**sample B06:** K_2E vacutainer (purple), 3 ml	**1** tube	_ _ _ _ _ _ _ _ _ _

Start time of centrifugation samples B03, B04: _ _. _ _ **hours**

Start time of centrifugation samples B01, B02: _ _. _ _ **hours**

Start time keeping at room temperature for 2 hours sample B05: _ _. _ _ **hours**

Freezing date and time samples: _ _ - _ _ - _ _ _ _ / _ _._ _ **hours**

B05 _ _._ _ **hours**

Samples processed by (name): _____

Warning: After centrifugation the specimen should be put upright into the rack in fridge or cool box. Samples should be handled carefully from this point on so as not to disturb the gel seal. Samples kept in a cool box should never come into direct contact with the ice pack, as this will damage them.

Attachment 2

Ordering information

Product	***Supplier***	***Article no.***	***Description***
Vacutainer SST II Advance, yellow cap	Becton Dickinson	367953	Serum, B01, B02
Vacutainer PPT K_2EDTA/Gel 15.8 mg	Becton Dickinson	362799	Plasma (EDTA), B03, B04
Vacutainer PAXgene Blood RNA	Becton Dickinson	762165	RNA, B05
Supplied by Erasmus MC			
BD K_2EDTA vacutainer, 3 ml, 13x75 mm, purple cap	Becton Dickinson	368856	Whole blood, B06
Sarstedt colour-coded cap Yellow	Sarstedt	65.716.002	Serum, B01a-b, B02a-b (wsch in 5ml cryovials)
Sarstedt colour-coded cap Violet	Sarstedt	65.716.008	Plasma (EDTA), B03a-b, B04a-b (wsch in 5ml cryovials)