FEAST v 2.0
17.08.2023

FEAST Project

Standard Operating Procedure

Blood collection, processing, handling, and storage procedures

1.1 Purpose

The purpose of the current SOP is to provide step-by-step instructions on the exact procedures that the research team needs to follow for conducting venous blood collection for biochemical analysis at baseline and follow-up examination.

1.2 General procedures for venous blood collection

Venous blood samples will be obtained from each participant for biochemical analysis following a 12-hour overnight fast, at baseline (T1), 12 weeks (T2) and 6 months (T3) (figure 1)

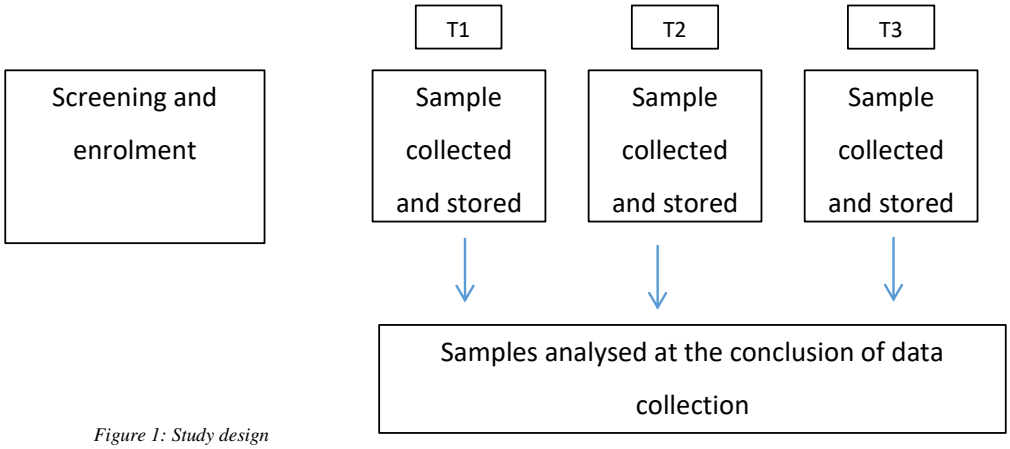

Figure 1: Study design

The researcher will perform venepuncture to obtain no more than 30mL of blood.

1.2.1 Consumables and supplies required for performing venepuncture

The consumables and supplies that will be used for performing the venepuncture in the study are the following:

- Disposable Latex gloves must be worn by the researcher and anyone else assisting with blood collection.
- Alcohol swab will be used to clean the venepuncture site.
- Winged steel needles appropriate for adults with an extension tube (a butterfly) will be used. The butterfly will have either a syringe or an evacuated tube with an adaptorSterile gauze pads will…..
- Adhesive hypo allergic bandages (plasters or Band-Aids) will be applied to the puncture site to minimize the risk of infection.
- Plastic Bag for Waste will be used to dispose all of the biohazardous waste generated as well as a sharps biocan to dispose of all needles.

1.2.2 Steps in obtaining venous blood from the participant

The steps for obtaining venous blood samples from the study participants are provided below:

Step 1: Complete general preparation.

- Find an indoor site to encourage privacy during blood collection. The site should have a table or other piece of furniture with a flat surface where you can lay out all consumables/ supplies. An examination bed should be readily available if the respondent feels faint and needs to lie down.
- Ensure that each subject has completed a 10-hour fast.

- Wash and dry hands, put on gloves before initiating blood collection from the participant.
- Take out a clean absorbent paper sheet and spread it over a flat surface to lay out consumable and supplies.

Step 2: Prepare the participant for the venepuncture.

- The individual should be seated comfortably in a chair with arm extended on the slanting armrest to form a straight line from the shoulder to the wrist. The arm and elbow should be supported firmly by the armrest and should not be bent at the elbow.
- Ask each volunteer if they have a history of fainting. If so, ensure that the blood sample is only drawn whilst the subject is lying down on a bed.
- Describe to the participant exactly what will be done during the collection of the blood sample.

Step 3: Prepare the venepuncture site.

- Apply (tighten) tourniquet.
- Ask the participant to close his/her hand so that the veins will become more prominent and thus easier to enter. Vigorous hand exercise or "pumping" should be avoided.
- Select the vein site. Palpate and trace the path of veins several times with the index finger. If superficial veins are not readily apparent, blood can be forced into the vein by gently massaging the arm from wrist to elbow. Several sharp taps at the vein site with index and second finger will cause the vein to dilate.
- Loosen tourniquet.
- The venepuncture site must be cleansed once with an alcohol swab to prevent any chemical or microbiologic contamination of either the patient or the specimen.
- Check equipment, tube selection and thread needle (or butterfly) securely onto tube holder (barrel).
- Re-apply the tourniquet and relocated vein position and direction. A tourniquet allows the veins to fill with blood, thus making the veins more prominent and easier to enter. Do not leave the tourniquet on for longer than 1 minute otherwise it may result in either hemoconcentration or variation in blood test values.
- Remove needle cover and check bevel is orientated uppermost.

Step 4: Blood drawing

- Puncture the skin 3–5 mm away from the vein; this allows good access without pushing the vein away.
- If the needle enters alongside the vein rather than into it, withdraw the needle slightly without removing it completely, and angle it into the vessel.
- Insert the tube into the holder and commence filling the tubes.
- Draw blood slowly and steadily.
- Release the tourniquet as soon as blood flow is established. Tourniquet release allows the blood circulation to return to normal and also reduces bleeding at the venipuncture site.
- Remove the tube from the holder and invert (8-10 times) to mix the blood with tube additives. Place blood samples on ice if required..
- Place a cotton wool above the venepuncture site, withdraw the needle and apply pressure.
- Dispose of needle in a sharps container.
- Check site and apply an adhesive bandage.
- Label all tubes immediately.

1.3 Blood processing and handling

1.3.1 Centrifuge procedure

Collected venous blood will be centrifuged and the extracted plasma and/or serum will be pipetted into aliquots according to the blood collection protocol.

- Set up in a well-ventilated environment, on a horizontally levelled and rigid surface with adequate load-bearing capacity.
- As safety zone maintain a clear radius of at least 30 cm around the centrifuge. Do not place any dangerous substances within this security zone.
- Open the centrifuge door by pressing the open button.
- Place the remaining tubes containing blood into appropriate sized adapters.
- Place the tubes containing water in opposite adapters, where they should mirror the placement of the tubes holding blood.
- Never place both tubes housing water and blood into the same adapters but should be placed in different adapters for even weight distribution.
- Place the adapters carefully and gently into the rotor buckets
- Seal the buckets with the lids and close the centrifuge.
- Use only with rotors which have been loaded properly.
- Make sure the rotor is locked properly into place before operating the centrifuge.
- Never overload the rotor.
- Never start the centrifuge when the centrifuge door is open.
- Do not lean on the centrifuge.
- Do not place anything on top of the centrifuge during a run.
- Gently close the centrifuge door. The centrifuge door mechanism will click and lock in place.
- Turn on the centrifuge by pressing the start button.
- Select the required speed and time from preprogramed setting or manually using the arrow keys (3000xg for 10 mins for each tube).

Once the centrifuge has completely stopped spinning wait for an audible sound and then open the centrifuge. Remove the tubes from the centrifuge and place them in a tube rack.

1.3.2 Handling of collected blood

Three different types of test tubes will be used per study participant to collect venous blood. The collected blood will be designated for whole blood, or plasma and serum separation. One 8ml EDTA tube (with added anticoagulant) will be used to collect whole blood for analysis, one 6ml heparin tube will be used for plasma extraction, and one 8.5ml SST tube will be used for serum extraction. Tubes will be labelled with study timepoint (T1, T2 or T3), participant ID, and type of sample. All information regarding blood collection tubes is presented in Table 1.

Table 1. Volume of blood in different test tubes

Test tube	**Blood volume**	**Designated for:**
EDTA tube	6 ml	Whole blood
Heparin gel tube	6 ml	Heparin plasma extraction
SST tube	8.5 ml	Serum extraction
Total blood:	22.5ml	

- The whole blood sample (6ml) collected in the EDTA tube will be stored at -80ºC, as indicated in Table 2.

Table 2. Volumes and use of EDTA whole blood sample.

Whole blood aliquot no.	EDTA volume	Designated for the analysis of:
1	6 ml	HbA1c

- The blood (8.5ml) collected in the SST tube will be left to separate at room temperature for 20 mins and then centrifuged at 3000 rpm for 10 min. The extracted (heparin) plasma will be pipetted into 4 aliquots of 1 ml (considering a 50% efficiency of centrifugation in plasma extraction). One aliquot of 1ml will be used for determining glucose, insulin, lipids, LFT and hsCRP, while the 3 aliquots of 1ml each will be stored at -80ºC, as indicated in Table 3.

Table 3. Volumes and use of SST plasma aliquots.

Plasma aliquot no.	EDTA plasma volume	Designated for the analysis of:
1	1000 µl	Glucose, insulin, lipids, LFT, hsCRP
		Designated for:
2	1000 µl	Storage at -80ºC
3	1000 µl	Storage at -80ºC
4	1000 µl	Storage at -80ºC

- The blood (6 ml) collected in the heparin tube will be centrifuged at 3000 rpm for 10 min and the extracted plasma will be pipetted into 3 aliquots of 600 µl (considering a 50% efficiency of centrifugation in plasma extraction). One aliquot of 600 µl will be used for determining cytokine concentrations, while the remaining 3 aliquots of 500 µl each will be stored at -80ºC, as indicated in Table 4.

Table 4. Volumes and use of heparin plasma aliquots.

Plasma aliquot no.	Heparin plasma volume	Designated for the analysis of:
1	1ml	Cytokines (IL-1β, IL-6, IL-8, IL-10, and TNF)
		Designated for:
2	1ml	Storage at -80ºC
3	1ml	Storage at -80ºC
4	1ml	Storage at -80ºC

NOTE: It is essential that ONLY NON-HAZARDOUS waste be placed in the wastepaper/ general rubbish bins. Pipette tips should be disposed in sharps containers, whereas laboratory and associated waste directly involved in specimen processing (i.e blood collection tubes, gloves etc) must be disposed in biological waste bags.

1.4 Blood storage

Eppendorf tubes or screw cap tubes must be clearly labelled with identification, media used and date, placed in a freezer well rack and should not be stored for long periods on a bench, but must be transferred with an ice esky box to a dedicated storage area (i.e. refrigerator, cold room or cupboard) as soon as possible.

Laboratory coats must be removed and hung up before leaving laboratory areas and should be laundered once a week. Hands must be washed with an antibacterial agent BEFORE leaving laboratory (Hibiclens/Microshield or equivalent, followed by extensive rinsing).