

## **SOPs on blood sample collection, processing and storage**

This document describes the SOP for the collection, processing, shipment (if needed) and storage of blood samples.

**A) Safety Considerations.** All blood specimens should be considered potentially infectious, the major risks being exposure to hepatitis and HIV. Universal precautions practices, including prior hepatitis vaccination, should be followed during blood collection and handling. All fresh and frozen biospecimens should be considered potentially infectious.

**B) Collection.** Collection of blood specimens should be carried out by trained personal to avoid compromising the quality or quantity of the sample. The study participants should receive clear oral and/or written instructions, with information, for example, about fasting and avoidance of medications as necessary for the planned analyses. For blood collection, standard protocols recommended by well-established organizations must be used as described below. A tube system (Becton Dickinson: Vacutainer; <http://www.bd.com>) with interchangeable plastic tubes should be used to collect blood.

Anticoagulated blood (consisting of plasma, buffy coat, and RBCs) is used for glycation analysis. There are several types of anticoagulants, which differ in their mechanism of action. EDTA chelates metals such as calcium and magnesium and will be used here.

**C) Shipment (when needed).** Depending on whether they are known to contain infectious agents and on the intended analyses, blood specimen shipments may be regulated as infectious substances or as diagnostic specimens. To properly classify the specimens to be included in a shipment, consult references provided in the International Society for Biological and Environmental Repositories Best Practices and by the International Air Transport Association.

Blood specimens are often exposed to temperature fluctuations during transit. The following are the temperature conditions required for the transport of blood. Here the samples will be sent frozen ( $-20^{\circ}\text{C}$ ) with gel packs designed for frozen temperatures ( $>3\text{ kg}$ ).

**D) Processing and storage.** Fractionation of blood results in the following components: (a) white blood cells; (b) erythrocytes; (c) platelets; (d) plasma.

If the fractions are not intended for immediate use, they should be aliquoted into small cryovials and stored in mechanical or liquid nitrogen freezers. Aliquoting is often necessary to preserve multiple samples to avoid thaw/refreeze cycles. The volume and number of aliquots should correspond to the intended analyses to avoid unnecessary long-term storage. Plasma will be stored at  $-80^{\circ}\text{C}$  within 1 hour of venipuncture and lymphocytes should be stored in the vapor phase of liquid nitrogen at  $-150^{\circ}\text{C}$ .

## **SOP for Blood Collection by Venipuncture**

This SOP describe the step-by-step method to obtain peripheral blood samples from adults needed for glycation testing, with proper specimen identification and handling, while ensuring patient and staff safety.

### **1 Materials and Equipment Required:**

Disposable gloves, alcohol swabs, tourniquet, vacutainer specimen tubes (EDTA), vacutainer holders, appropriate size sterile disposable needles, cotton balls/swabs, sharps disposal container, refrigerator and centrifuge machine.

### **2 Procedure:**

2.1 All required materials for blood drawing should be assembled before performing the procedure.

2.2 New sterile, single use needles and vacutainer tubes are to be used for each blood draw, and after completion needles must be properly disposed of in a puncture resistant container. They are never to be cleaned and reused for any purpose.

2.3 Check the specific Lab requisition form to confirm the quantity of blood to be drawn and which kind of vacutainer tubes to use.

2.4 Vacutainer tubes should be labeled by patient identification number, patient's name etc. before sample collection. Be sure to verify the identity of the client and clinic number before labeling the tubes. Do not prepare tubes for more than one subject at a time.

2.5 The personal should wear disposable gloves and use aseptic technique. Gloves should always be worn when handling or transporting specimens if there is any possibility of direct contact with blood.

2.6 Seat ambulatory patients in a comfortable chair with the extremity from which blood will be drawn supported on a sturdy table or other support. The preferred site is the median ante-cubital vein of the upper extremity. A tourniquet may be used to transiently distend veins prior to drawing blood. Do not leave the tourniquet too long.

2.7 Using the tip of the index finger, examine and feel the vein, and decide exactly where to place the puncture.

2.8 Disinfect the site by swabbing the skin in small outward circles with an alcohol swab. Do not touch the prepared puncture site with your fingers after disinfecting the skin.

2.9 Using aseptic technique, insert the needle of the vacutainer device into the vein. If possible, always allow the full amount of blood to be drawn by each evacuated tube when using the vacutainer system tubes. After drawing, mix the blood in the EDTA tubes by inverting the tubes several times.

2.10 After drawing the required blood samples, release the tourniquet. Remove the needle from the vein, cover the puncture site with a cotton swab, and hold until adequate haemostasis is visible.

2.11 Insert the tube in the centrifuge for 10min at 4° C at 2500g.