# Standard Operation Procedure (SOP) for Biobanking Sampling Procedure – Manual Use

## 1. Plasma, Serum and PAXgene Blood Collection

**1.1. Equipment and reagents to be supplied by user**
**1.1.1. For blood withdrawal**
**1.1.2. For following procedures**

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**1.3. Serum collection protocol**

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## 2. Preparation of a Dried Blood Spot Sample (Archive sample for back-up)

**2.1. Equipment and reagents to be supplied by user**

**2.2. Dried blood spot protocol**

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# 1. Plasma, Serum and PAXgene Blood Collection

## 1.1. Equipment and reagents to be supplied by user

### 1.1.1. For blood withdrawal

- 1 BD Vacutainer® Safety-Lok™ Blood Collection Set, 21G x ¾" x 7" (0.8 x 19 mm x 178 mm), Becton Dickinson, REF 367282
- 1 Standard Tube Holder, Greiner Bio-One, REF 450201
- 3 BD Vacutainer® K2E (EDTA), Becton Dickinson, REF 367525
- 1 BD Vacutainer® CAT (Clot Activator Tube), Becton Dickinson, REF 367896 (Do not use gel or serum separator tubes for drug levels as the drug may be absorbed into the gel)
- 1 PAXgene® Blood RNA Tube from PreAnalytiX GmbH, REF 762165

### 1.1.2. For following procedures

- Refrigerated centrifuge with swinging bucket rotor and tube carriers/adapters for 16 x 125 mm tube size, capable of running at 1,100 x g and 4°C
- Cooling rack, IsoFreeze MCT-Rack, Kisker, REF KR-24 or wet ice
- 1000 µL Pipette
- 1000 µL Filter Tips
- 14 Protein LoBind Tubes, Protein LoBind, 2.0 mL, PCR clean, Eppendorf, REF 0030108132
- Dulbecco´s Phosphate Buffered Saline (DPBS, 1x), gibco® by life technologies™, REF 14190-094

For complete biobanking set a blood withdrawal order is recommended as follows:
1. and 2. Tube EDTA for archive sample and DNA extraction, 3. Tube EDTA for Plasma, 4. Tube CAT for Serum, 5. Tube PAXgene for RNA. If the PAXgene® Blood RNA Tube is the only tube to be drawn, blood should be drawn into a "Discard Tube" prior to drawing blood into the PAXgene® Blood RNA Tube so the interior volume of the blood collection set used during phlebotomy can be primed. Otherwise the PAXgene® Blood RNA Tube should be the last tube drawn in the phlebotomy procedure.

## 1.2. Plasma collection protocol for EDTA tube no. 3

1. After blood withdrawal, mix the tube by inverting several times to ensure the correct mixing of blood with EDTA.

2. Store tube at 4°C and process as quickly as possible (within 30 minutes).

3. Centrifuge for 15 minutes at 1,100 x g at 4°C to pellet red and white blood cells.

4. Immediately after centrifugation, transfer plasma (supernatant) to Protein LoBind Tubes **in a cooling rack or on ice**. Contact with, or disturbance of, the pellet must be avoided at all costs during this transfer. Aliquot 0.5 mL plasma into up to 7 x 0.5 mL aliquots in LoBind 2.0 mL tubes. If more plasma is available fill up the last aliquot with the rest of it.

5. Place the aliquots straight into -70°C freezer.

6. Store frozen aliquots until analysis or shipment at -70°C. For clinical sites which do not have a -70°C freezer the aliquots should be stored temporarily in a -20°C freezer before their shipment to a -70°C facility. Ideally, -20°C storage should be no longer than 2 weeks, and absolutely no longer than 4 weeks, before shipment to a -70°C facility. Make sure that you ship the aliquots in large quantity of dry ice to avoid thawing and avoid freeze-thaw cycles.

7. If the EDTA blood is needed for further analyses, e.g. DNA isolation, the blood tube can be filled up with 1 x DPBS. After mixing the pelleted cells with DPBS, the DPBS blood can be frozen at -20°C or for long-time storage at -70°C.

## 1.3. Serum collection protocol

1. After blood withdrawal, mix the tube by inverting several times to ensure the correct mixing of blood with the clot activator.

2. Incubate at room temperature until clotted, at least 1 hour (maximum 2 hours).

3. Centrifuge for 15 minutes at 1,100 x g at 4°C to pellet the clotted blood.

4. Immediately after centrifugation, transfer serum (supernatant) to Protein LoBind Tubes **in a cooling rack or on ice**. Contact with, or disturbance of, the pellet of the clotted blood must be avoided at all costs during this transfer. Aliquot 0.5 mL serum into up to 7 x 0.5 mL aliquots in LoBind 2.0 mL tubes. If more serum is available fill up the last aliquot with the rest of it.

5. Place the aliquots straight into -70°C freezer.

6. Store frozen aliquots until analysis or shipment at -70°C. For clinical sites which do not have a -70°C freezer the aliquots should be stored temporarily in a -20°C freezer before their shipment to a -70°C facility. Ideally, -20°C storage should be no longer than 2 weeks, and absolutely no longer than 4 weeks, before shipment to a -70°C facility. Make sure that you ship the aliquots in large quantity of dry ice to avoid thawing and avoid freeze-thaw cycles.

7. Discard the blood tube with the pelleted clotted blood.

### 1.4. PAXgene® blood collection protocol

1. After blood withdrawal, mix the tube by inverting 8 to 10 times to ensure the correct mixing of blood with stabilizing solution.

2. Incubate the PAXgene® Blood RNA Tube upright at room temperature (18-25°C) for a minimum of 2 hours and a maximum of 72 hours (3 days) before processing or transfering to refrigerator (2-8°C) or freezer (-20°C). **Do not freeze tubes upright in a styrofoam tray as this may cause the tubes to crack.**

3. The PAXgene® Blood RNA Tube can be stored at -20°C and below. If tubes are to be kept at temperatures below -20°C, freeze them first at -20°C for 24 hours, then transfer them to -70°C or -80°C.

# 2. Preparation of a Dried Blood Spot Sample (Archive sample for back-up)

### 2.1. Equipment and reagents to be supplied by user

- EDTA blood from Tube no. 1
- 1 x 1000 µL Pipette
- 1 x 1000 µL Filter Tip
- Pre-Cutted piece of Whatman paper (3 x 7 cm), GE Healthcare by Sigma Aldrich, REF 3030917
- 1 Sample bag (5 cm x 8 cm), Carl Roth, REF P277.2

### 2.2. Dried blood spot protocol

1. Drip three drops of EDTA blood onto the center of the paper (circa 300 µL).
2. Let the dropped blood dry for about 3 days at room temperature on a metal stand (figure 1).

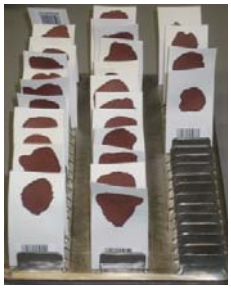
Figure 1.

3. Afterwards, pack the archive sample into a sample bag (figure 2).


Figure 2.

4. Store the archive sample in a plastic box at room temperature.