SOP.BIO.001 - WHOLE BLOOD PROCESSING

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STANDARD OPERATING PROCEDURES

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1. SCOPE

This Standard Operating Procedure defines the handling, processing and freezing protocols of whole blood samples and derivates, which will be preserved at Biobanco.PT.

2. SAFETY INFORMATION

All specimens should be treated as infectious and handled according to "standard precautions". Blood must be processed only by trained staff. Lab coat, gloves, safety goggles and other individual protection devices must always be worn while collecting and handling samples.

A. Interferences

In order to avoid any alteration of the sample, the sample should be processed as soon as possible and preferably within 30-60 minutes from collection. During this time, samples must be preserved at 2-8 $^{\circ}$ C.

The samples must be processed between 24h and 48h from collection if not otherwise specified.

3. EQUIPMENT / INFRASTRUCTURES REQUIRED

• Collection tubes (eg EDTA or heparin lithium for whole blood conservation, plasma separation and DNA extraction);

- Collection tubes (eg gel without anticoagulant for serum separation);
- -80 ° C ultrafreezers;
- Pipettes;
- Sterile tips;
- 1-2ml sterile cryovials with a screw cap;
- Cryobox;
- Disposable gloves
- Centrifuge with sealed buckets or sealed rotor;

4. DESCRIPTION / PROCEDURE

4.1. Specimen identification

Samples must be identified at the time of harvest, labeled and treated so that they respect the right of donor privacy in accordance with the law n.° 12/2005, published in Diário da República. Each tube must be labeled with code with the identification number generated by the software, which ensures the traceability of the sample and separation of personal and medical data.

4.2. Processing

i) Check that all specimens and relative documentation are available and record relevant information; If any documentation is missing, samples should be placed in the freezer "Quarantine" and the technical manager of the Biobank should contact the unit or person responsible for the samples.

ii) Register samples in the computer system as well as the relevant data. A label will be generated automatically with a code.

iii) Processing and freezing, according to the sample specific requirements

4.2.1 Whole Blood separation and freezing:

i) Invert the tube gently about 5 times; excess inversion can cause changes in the integrity of the sample.

ii) Transfer the whole blood for a cryovial of 2ml with thread, already properly labeled and aliquot the appropriate volume. (It is recommended 2 1ml aliquots)

iii) Seal the cryovial and place in the -80 °C ultrafreezer on the place automatically generated by the software.

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4.2.2 Serum separation and freezing

i) Invert the tube gently about 5 times, the reversal in excess can cause alterations in the integrity of the sample.

ii) Ensure that the centrifuge is in good conditions and that the tubes are properly closed and balanced to avoid breakage and spilling.

iii) Centrifuge at 800g for 10 minutes at room temperature, with brake.

iv) Remove the tubes carefully from the centrifuge.

v) Recover the serum aseptically using a pipette and disposable sterile tips. Transfer the serum into labeled 2 ml sterile screw cap cryovials (It is recommended 6 250ul aliquots); secure the cap tightly and transfer into the -80°C freezer for long-term storage.

4.2.3 Plasma separation and freezing

i) Invert the tube gently about 5 times, the reversal in excess can cause alterations in the integrity of the sample.

ii) Ensure that the centrifuge is in good conditions and that the tubes are properly closed and balanced to avoid breakage and spilling.

iii) Centrifuge at 800g for 10 minutes at 4°C with brake.

iv) Remove the tubes carefully from the centrifuge.

v) Recover the serum aseptically using a pipette and disposable sterile tips. Transfer the plasma into labeled 2 ml sterile screw cap cryovials (It is recommended 2 aliquots of 500ul); secure the cap tightly and transfer into the -80°C freezer for long-term storage.

A. Quality control:

All equipment used, such as pipettes, centrifuge and freezers should be checked, cleaned and disinfected regularly according to the manufacturer's recommendations

B. Backup

It is recommended to split stored biospecimens into two sets of aliquots, each set stored at a different location; this strategy will avoid loss in case of adverse events in one location.

5. RECORDS

Records' Identification	Indexation	Archive Responsible

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