Institute of Health Carlos III

Red Nacional de Biobancos
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SOP
Plasma

Blood Products Working Group

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Collection, Processing and Storage of Plasma Samples

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1. ABBREVIATIONS

- **EDTA**: Ethylenediaminetetraacetic acid.
- **ACD**: Acid citrate dextrose.
- **PBMCs:** Peripheral blood mononuclear cells.

2. DEFINITIONS

- **Blood plasma:** liquid and non-cellular fraction of the blood.
- **Pellet**: white little ball of cells (although sometimes it may have a reddish color due to the presence of some erythrocytes). The presence of erythrocytes must be avoided, but it is not sufficient reason to discard the sample.
- **EDTA:** Ethylenediaminetetraacetic acid. Anticoagulant agent that blocks the blood coagulation cascade by attracting ionic calcium.
- **ACD:** anticoagulant solution of dextrose, sodium citrate and citric acid that inhibits coagulation by attracting ions.
- **Heparin**: anticoagulant agent that prolongs blood clotting time by activation of antithrombin III.
- **Lipemia**: the presence of lipids in the blood. The plasma takes on a cloudy or milky appearance.
- **Jaundice:** binding of bilirubin to plasma albumin, leading to intense yellow plasma.
- **Hemolysis**: destruction of erythrocytes causing the plasma to have a pink or red color.

3. OBJECTIVE

The objective of this procedure is to define the course of action and to establish the basic quality guidelines with respect to collecting and handling and to processing of plasma samples that will be deposited in biobanks belonging to any center or hospital affiliated to the National Biobank Network.

4. SCOPE

This procedure applies to all plasma samples that are obtained in order to be stored in a biobank. This protocol does not detail the occupational health and safety processes regarding biohazardous materials and/or chemical products, and it is recommended that the personnel follow the Health and Safety rules established in each center.

5. MATERIALS

- *Courier Service holding a permit for the transport of biological materials:*

Material	*UN Classification*		*Packing instructions*				*Comments*
	Class	*No.*	*ADR*	*RID*	*ICAO*	*IMDG*	
Infectious samples affecting humans	*6.2*	*2814*	*620*	*620*	*692*	*620*	*Materials groups 2,3,4*
Diagnostic specimens	*6.2*	*3373*	*650*	*650*	*650*	*----*	*Materials groups 1,2,3*

- *For non-infectious samples: Bag or container for internal transport in the hospital.*
- *For infectious or hazardous samples: Transport container for dangerous substances that comply with the effective legislation: Royal Decree 664/97, following "Packing Instruction 620 (IATA - ICAO 602)"*
- *Syringes and/or material required for collecting blood.*
- *Vacutainer-type venous blood collection tube containing anticoagulant (K_2 or K_3 EDTA, citrate or heparin)*
- *Blood collection tube racks*
- *Centrifuge with adapters suitable for the type of collection tubes used (5 and 10ml tubes, and 15 or 50 ml Falcon tubes)*
- *Gloves for protection during handling*
- *Sterile 1-5 milliliter Pasteur pipettes*
- *Pipettes (to collect volumes between 0.2 and 1 milliliter)*
- *Sterile tips with or without filter that fit the type of pipettes used*
- *15 ml Falcon tube with screw cap*
- *Sterile cryotubes (from 0.5 to 2 milliliters)*
- *Cryotube racks*
- *Cryo storage boxes*
- *Labels appropriate for the type of cryotubes*
- *Printer for labeling samples*
- *-80º C ultra-low temperature freezer with temperature recording system and temperature maintenance system in case of power failure (CO2 injection, internal Energy Management System (EMS), generator) and telephone alarm system*
- *Sample management software (Maxwell, Bio-e Bank, NorayBanks, etc.)*

6. DEVELOPMENT

6.1. PRIOR CONSIDERATIONS

- **Choice of anticoagulant:** If the whole blood sample is taken for a specific purpose, it is recommended to choose the type of anticoagulant depending on the type of study/analysis that will be done with the derived samples.
  - K_2 or K_3 EDTA: given their characteristics, they are not recommended for obtaining plasma samples in which tests are to be done that measure the presence of ions or that include divalent cations as reaction intermediates. Conversely, it should be used when the blood sample was obtained for PBMCs or the cell pellet.
  - ACD: due to its characteristics it not recommended for obtaining plasma samples in which immunoassays will be done, since it decreases the expected values. Its use is recommended if the blood sample was obtained for obtaining erythrocytes.
  - Heparin: because of its characteristics it is not recommended for plasma in which peptide or proteomic analyses are going to be done, because it can interfere with some mass spectrometric analyses. Its use is recommended if the sample was obtained for cellular studies.
- **Time:** It is recommended to adjust the optimum processing time of the sample to the type of test that will be done with the sample or its derivatives. According to experience, the maximum recommended time is:
  - For studies of biomarkers in plasma, it is advised to centrifuge the blood as quickly as possible (ideally within 30 minutes after extraction) to prevent alterations in the plasma composition due to changes in expression that occur in blood cells as a result of hypoxia.
  - For cell assays: 1.5 hours
  - For virological studies: 24 hours
- **Temperature:** To determine the temperature that minimizes alteration of the sample, the most important factor to take into account is the estimated time that processing will take (see section 6.3.2).
- **Number of centrifugations:** It is estimated that at least 14% of the peptides that make up the plasma are derived from platelets due to post-extraction activation or to the existence of residual platelets after centrifugation of the blood. The best method to reduce the number of platelets in plasma to <10/nL is to do a double centrifugation. In any case, the requirements of the study for which the sample was taken must be taken into account and the SOP must be adapted accordingly, if necessary, as follows:
  - As a general recommendation and for measuring free plasma biomarkers, it is advisable to remove platelets and minimize the release of their contents by double centrifugation. This has been taken into consideration in this SOP.
  - In exceptional cases, when measuring biomarkers sequestered by platelets or in specific hematology and cardiology studies related to platelet function, centrifugation conditions must be adapted to the requirements of the study.
- **Storage time:** It is advisable to be aware of the time and temperature at which the samples have been stored, since many components of the plasma are unstable even at -80ºC and become undetectable over time.
- **Data recording:** It is very important to note and record the values of the preanalytical variables considered at this point so that the samples are documented well. Thus, the values of the search parameters can be restricted to the needs of the study for which the sample was requested, which allows us to select more homogeneous samples that give more reproducible results.

6.2. RECEIPT OF THE SAMPLE IN THE BIOBANK

6.2.1 Check the information and identification of the tubes and ensure the correct relationship between tubes and patient information, in accordance with the confidentiality commitment required by the Data Protection Act. Label and record the sample according to the sample management procedure used by the center.

6.2.3 It is advised to gather the maximum amount of information possible concerning the sample, both at the time of reception and after processing and storage, and depending on the studies for which they will be used, for example:

- Date and time of receipt and/or processing
- Transport conditions until receipt in the biobank: temperature and time elapsed since its extraction.
- Volume of blood received
- Types of tube and anticoagulant
- Degree of hemolysis
- Degree of Lipemia
- Degree of jaundice
- Degree of Coagulation
- Processing time
- Date and time of freezing

6.2.4 Register the arrival of the sample and associated data in the computer system.

6.3. COLLECTION OF PLASMA AND STORAGE OF ALIQUOTS

6.3.1 Start from whole blood collected by venipuncture. Processing whole blood to obtain plasma consists of separating the cellular fraction from the blood in order to obtain plasma aliquots that are as representative as possible of the physiological state of the donor at the time it was extracted, and storing them under Biobank conditions to avoid interference in the diagnostic analyses that are to be carried out.

6.3.2 **Temperature:**

- If the blood sample is going to be centrifuged immediately (within 30 minutes after extraction) it is advisable to keep it at room temperature (16-24ºC) until processing, to minimize platelet activation occurring at low temperatures that causes the release of proteins that irreversibly alter the plasma composition.
- If we know that the sample is going to be processed later it is advisable to keep it refrigerated (2-6ºC) until processing, in order to prevent degradation of temperature-sensitive components of the sample.

6.3.3 **Processing time:**

It is recommended that the time between blood collection and freezing is **less than 2 hours**, minimizing as much as possible delays until the first centrifugation. The maximum acceptable time for certain studies is up to 72 hours, if the sample has been stored at 4ºC.

6.3.4 Centrifuge the sample at **1300-1500g for 10 minutes**. To select the temperature, follow the same criteria as outlined in paragraph 6.3.2.

6.3.5 With this first centrifugation plasma is separated from the cellular blood fraction. Three phases will be seen in the tube:

- The upper phase is clear, transparent and yellow, and corresponds to the plasma.
- The intermediate phase, which is very thin and light gray, contains the leukocytes.
- The bottom phase is dark red and corresponds to the erythrocytes.

6.3.6 Aspirate the plasma (yellow upper phase) carefully with a pipette and transfer it to a properly identified/labeled sterile 15 ml tube.

6.3.7 Centrifuge the transferred material at **2500g for 15 min** at the same temperature as used in paragraph 6.3.4. This second centrifugation eliminates most of the platelets contained in the plasma.

6.3.8 Divide into aliquots of at least 0.5 ml in suitable cryogenic vials that are properly labeled and identified. Close the tubes properly to obtain an airtight seal. Record the number of aliquots obtained for each sample. Store within 2 hours. After obtaining the platelet-poor plasma, it should be kept at 4ºC; otherwise, it must be stored immediately at -80ºC.

6.3.9 Record the location of the sample, as well as the information detailed in section 6.2.3, in the sample management software used by the biobank.

7. REFERENCE DOCUMENTATION

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8. RELATED DOCUMENTATION

- *SOP for blood extraction*
- *Data collection sheet associated with the sample*
- *SOP quality control*

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