Institute of Health Carlos III

Red Nacional de Biobancos
Spanish National Biobank Network

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SOP
Peripheral Blood Mononuclear Cells

Blood Products Working Group

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Collection, Processing and Storage of Peripheral Blood Mononuclear Cell (PBMC) Samples.

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Mª Ángeles Muñoz

Coordinator of the Working Group

Manuel M Morente

Coordinator of the National Biobank Network - ISCIII

National Biobank Network - ISCIII

www.redbiobancos.es

AUTHORS.

This Code of Best Practices was prepared by the **Blood Products Working Group** (www.redbiobancos.es):

Maribel García Sánchez, Hospital Virgen Macarena

Lina Mayorga, Hospital Carlos Haya

Tatiana Díaz, Hospital Carlos Haya

Inmaculada Martín, Hospital Carlos Haya

Pilar Giraldo Castellanos, Hospital Miguel Servet

Fernando Civeira Murillo, Hospital Miguel Servet

Miguel Pocoví Mieras, Hospital Miguel Servet

Pablo Isidro Marrón, Central University Hospital of Asturias

Jacobo Martinez, Center for Public Health Research (CSISP)

Inés Santiuste, Hospital Marqués de Valdecilla

José Manuel González de Buitrago, University Hospital of Salamanca

Edurne Pedrosa, Health Sciences Research Institute of the Germans Trías i Pujol Foundation

Alfonso Monje Hernández, San Juan de Dios. Mental Health Services

Gerard Pardo, Hospital Dr. Josep Trueta

Beatriz Bellosillo, Hospital del Mar

Luis Gallart Millán, Hospital Joan XXIII

Anna Bosch, Clinical and Provincial Hospital of Barcelona - IDIBAPS

Nieves Doménech García, La Coruña University Hospital

Mª Ángeles Muñoz Fernández, Hospital Gregorio Marañón

Almudena García Torres, Hospital Gregorio Marañón

Irene Consuegra, Hospital Gregorio Marañón

Rosario Martínez Marín, Hospital Virgen de La Arrixaca

Mª Antonia Fortuño Cebamanos, University Clinic of Navarre

Isabel Gil Aldea, Hospital of Navarre

Inés Aroca Siendones, San Cecilio University Hospital, Granada

Clara Rodríguez, Basque Biobank/Basque Transfusion Center

Coordinator:

Mª Ángeles Muñoz, Hospital Gregorio Marañón

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1. ABBREVIATIONS

DMSO: Dimethylsulfoxide (CH_3SOCH_3) is a colorless organic liquid containing Sulphur; it is used as an organic solvent. Cryopreservative agent. It is a low-molecular-weight and membrane-permeable compound. It acts by displacing the water within the cell, avoiding the formation of ice crystals during freezing.

RPMI: culture medium originally formulated to maintain fibroblasts in suspension; today it is used for the maintenance of numerous cell lines and hybridomas.

PBS: Phosphate buffered saline

EDTA: Ethylenediaminetetraacetic acid. EDTA attracts calcium ions, thus blocking the coagulation cascade (dipotassium salt K2, or K3).

ACD: Citric acid, citrate and dextrose in amounts of 0.9, 2, and 2 g, respectively, in 120 ml of distilled water. It is used to obtain plasma for coagulation and platelet function assays. It is used in collection and storage for transfusion since it preserves the blood longer, particularly the survival of erythrocytes: 21-32 days-70% survival. It changes the calcium concentration.

CPD: Citrate - Phosphate - Dextrose.

CPD-A: Citrate - Phosphate - Dextrose - Adenine

FBS: Fetal bovine serum

2. DEFINITIONS

PBMCs: Peripheral blood mononuclear cells (monocytes and lymphocytes)

Granulocytes: Granulocytes are polymorphonuclear leukocytes that are characterized by the way their cytoplasmic granules are stained under light microscopy. There are three types of granulocytes in human blood:

Neutrophils: are stained with neutral dyes or are hardly stained

Eosinophils: strong reddish staining

Basophils: show affinity for basic dyes and acquire a bluish 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 the processing of whole blood 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 PBMC samples that are obtained in order to be processed and 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 AND SERVICES

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

Material	*UN Classification*		*Packing instructions*				*Comments*
	Class	*No.*					
Infectious samples affecting humans	*6.2*	*2814*					*Materials groups 2, 3, 4*
Diagnostic specimens	*6.2*	*3373*					*Materials groups 2, 3, 4*

For non-infectious samples: Bag or container for internal transport in the hospital.

For infectious or hazardous samples: Transport container for dangerous substances that complies with the effective legislation: Royal Decree 664/97, following "Packing Instruction 620 (IATA - ICAO 602)"

Syringes and/or material required for collecting blood.

15 ml Leucosep tubes or 15 ml sterile tubes

50 ml Falcon-type tubes

Sterile cryovials

Sterile pipettes and filter tips

Identification labels

PBS

Ficoll

Complete RPMI (RPMI 1640 glutamine/Hepes + 15% FBS + 1% antibiotic)

DMSO freezing medium (FBS + 7.5-10% DMSO)

6. DEVELOPMENT

6.1. PRIOR OPERATIONS

6.1.1. Blood must be collected after the patient has signed the informed consent for donating samples to the biobank. It is recommended that the time between blood collection and freezing at -80ºC be defined based on the type of studies for which the sample is intended. Thus, based on preliminary tests, it has been determined that: a) optimum time for cell studies: maximum 1.5 hours after extraction; and b) optimum time for virological studies: maximum 24 hours after extraction.

6.1.2. Blood is collected via peripheral venipuncture. The people in charge of carrying out this procedure and of programming extractions must coordinate with biobank staff to ensure that the blood collection tubes with anticoagulants are properly identified and that a proper collection and reception of the sample is guaranteed.

6.1.3. The type of anticoagulant used must be the most appropriate one for studies for which the sample is intended. Based on previous experience, the following anticoagulants are advised: a) Cell proliferation studies - leukocytes: Heparin. b) DNA extraction: EDTA. c) Cryopreservation of red blood cells: ACD, CPD-A or CPD

6.1.4. It is advised to take the maximum possible information concerning the sample at the time of extraction:

Date and time of withdrawal.

Type of anticoagulant.

Incidents not related to the protocol.

6.2. VERIFICATION AND IDENTIFICATION OF THE TUBES

Check patient information, while always maintaining privacy and ethics as guaranteed by Law 15/1999, of December 13, on the Protection of Personal Data, and other relevant national laws applicable to this process, and ensure the correct relationship between the properly labeled blood collection tubes and patient information.

6.3. COLLECTION OF PERIPHERAL BLOOD MONONUCLEAR CELLS USING LEUCOSEP TUBES

Optional: Pool the contents of all blood collection tubes into one 50ml Falcon tube to homogenize the blood.

6.3.1 Add 3.2 ml of Ficoll to each labeled Leucosep tube (empty tubes)

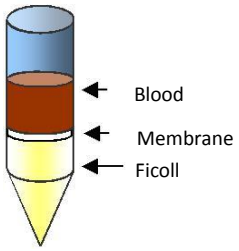
6.3.2 Centrifuge the Leucosep tube containing Ficoll at 1000xg for 1 min. Remove excess Ficoll by decanting or pipetting.

6.3.3 Centrifuge the tubes with blood at 1500xg for 15 min.

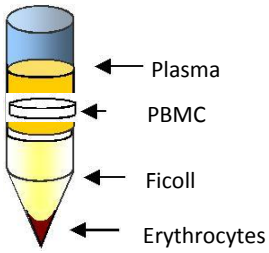
6.3.4 Aspirate the plasma without taking mononuclear cells, which form a white layer above the erythrocytes and the granulocytes, and discard or aliquot as described in SOP "Collection of plasma".

6.3.5 Aspirate 2 ml of the mononuclear cell layer and transfer to a 15 ml Falcon tube containing 4 ml PBS. Invert the tube to mix the solution well.

6.3.6 Add the mononuclear cell layer with the PBS or the blood to the Leucosep tube of point 6.3.1.



6.3.7 Centrifuge the tubes at 800xg for 15-30 min. at 18-25ºC without using the brake



Continue with point 6.5

6.4. COLLECTION OF PERIPHERAL BLOOD MONONUCLEAR CELLS WITHOUT USING LEUCOSEP TUBES

Optional: Pool the contents of all blood collection tubes into one 50 ml Falcon tube to homogenize the blood.

6.4.1 Add one volume of Ficoll for each two blood volumes (Ficoll: Blood 1: 2)

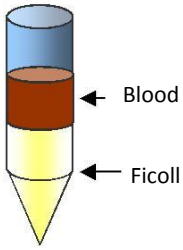
6.4.2 Centrifuge the tubes with blood at 1500xg for 15 min. Optional: homogenize the blood and proceed with step 6.4.5

6.4.3 Aspirate the plasma without taking mononuclear cells, which form a white layer above the erythrocytes and the granulocytes, and discard or aliquot as described in SOP "Collection of plasma". Optional: add PBS in an equal amount as the volume of plasma removed.

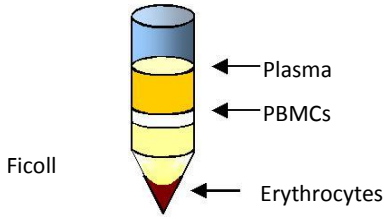
6.4.4 Mix the blood well with the PBS and continue with step 6.4.5.

6.4.5 Aspirate 2 ml of the mononuclear cell layer and transfer to a 15 ml Falcon tube containing 4 ml PBS. Invert the tube to mix the solution well.

6.4.6 Add the mononuclear cell layer with the PBS or the blood on top of the Ficoll, keeping the tube as horizontally as possible to avoid mixing of Ficoll and blood.



6.4.7 Centrifuge the tube at 500xg for 10 min. at 18-25ºC without using the brake.



Continue with point 6.5

6.5. COLLECTION OF PERIPHERAL BLOOD MONONUCLEAR CELLS

6.5.1 Remove the white layer of PBMCs and transfer it to a sterile tube. Make up the volume with PBS.

6.5.2 Centrifuge the tube at 500xg for 10 min. at 18-25ºC.

6.5.3 Remove the supernatants of the tubes by decanting, taking care not to break the cell pellet. Resuspend the obtained pellet in PBS.

6.5.4 Remove a 10-25 µl sample and count cells.

6.5.5 Centrifuge the tube at 500xg for 10 min. at 18-25ºC.

6.5.6 Discard the supernatant and resuspend pellet in complete medium:

For culture (RPMI+ 10 % FBS)

For freezing (FBS+7.5 - 10 % DMSO)

6.5.7 Make the appropriate dilution to achieve a desired concentration:

For freezing 7-15 x 10^6cells/ml.

6.5.8 Freeze or place in culture.

6.6. MAINTAINING TRACEABILITY AND DATA ASSOCIATED TO A SAMPLE:

Biobanks advise to gather the maximum amount of information possible concerning the sample, both at the time of receipt 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
- Degree of hemolysis
- Volume of blood received
- Degree of lipemia
- Degree of jaundice
- Degree of coagulation
- Incidents during processing

7. REFERENCE DOCUMENTATION

- *Standard ISO 9001:2008. Quality management systems. Requirements.*
- *Standard ISO 6710 which establishes the color code for tubes according the anticoagulant used.*

8. RELATED DOCUMENTATION

- *Isolation of Whole mononuclear cells from peripheral blood and cord blood: Kanof ME, Smith PD, Zola H. Curr Protoc Immunol 2001 May Chapter 7, unit 7.1*

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