

TÍTULO: BLOOD PLASMA COLLECTION, PROCESSING AND STORAGE		
ARCHIVADO: PNT_8.5.001_v1.1_EN_PLASMA_COLLECTION_PROCESSING_STORAGE		Nº DE CÓDIGO: 8.5.001
VERSIÓN: 1.3	FECHA DE EDICIÓN: 26/7/2017	PÁG. 1 DE 4

REVISADO POR: Estefanía Piñero, Víctor Miguel Galvez, Laura Vidaña, Daniel Pons FECHA: 24/07/2017	FIRMA:
APROBADO POR: Cristina Villena FECHA: 26/7/2017	FIRMA:

1. OBJETIVE

The aim of this procedure is to guarantee safety, timely and efficient blood plasma sample preservation from patients that have signed the informed consent form and agree with the participation in the Pulmonary Biobank Consortium.

2. SCOPE

This procedure describes how to collect, to process and to store blood plasma samples. This protocol does not detail occupational safety and health regarding biohazard material and/or chemicals processes. It is recommended that the staff follows the established Health and Safety rules of each center.

3. RELATED DOCUMENTS

Not applicable

4. ROLES AND RESPONSIBILITIES

The fulfilment of this standard protocol rests with all the members of the Pulmonary Biobank Consortium who are responsible for management, processing and storage of blood plasma.

5. MATERIALS AND EQUIPMENT

Recommended materials and equipment are listed below. Depending on the place where the task or procedure is performed, these materials may be replaced by alternative or equivalent products.

Materials and Equipment	Materials and Equipment (specific to center)
Bag or container for transportation in hospital	
Blood extraction tube for hematology (with anticoagulant EDTA-K ₃)	

TÍTULO: BLOOD PLASMA COLLECTION, PROCESSING AND STORAGE	
ARCHIVADO: PNT_8.5.001_v1.1_EN_PLASMA_COLLECTION_PROCESSING_STORAGE	Nº DE CÓDIGO: 8.5.001

Centrifuge with blood extraction tubes adapter	
Sterile Pasteur pipettes	
Rack for blood extraction tubes	
Rack for cryotubes	
1,5-2ml sterile cryotubes	
Pens and markers	
Gloves to protect staff when manipulating blood and/or biohazard material	
Filter paper	
Lab coat for protection against spills and splashes	
Adequate and suitable labels for collection tubes	
Adequate and suitable labels for cryotubes	
Labeling printer	
Cryostorage boxes	
-80°C ultrafreezer	

6. GLOSSARY

Blood plasma: Acellular liquid fraction (extracellular matrix) of blood. It consists of 90% water and other dissolved components, mainly proteins. It also contains carbohydrates, lipids and waste products from metabolism. It is the major component in blood accounting for approximately 55% of total blood volume. Blood cells represent the other 45%, related to hematocrit.

EDTA: Ethylenediaminetetraacetic acid. EDTA attracts calcium ions blocking coagulation cascade.

7. PROCEDURES

7.1 COORDINATION FOR BLOOD SAMPLE EXTRACTION AND PROCESSING

1. Blood must be collected before surgery and close to the day when lung tissue extraction is performed. Time between blood extraction and freezing at -80°C should not exceed 45min. if a proteomic study with plasma will be performed.
2. The designated person, who will carry out this procedure, knowing the schedule of surgery, will coordinate with the qualified staff ensuring properly tubes identification and appropriate sample extraction.

7.2 TUBE VERIFICATION AND IDENTIFICATION

1. Verify patient information ensuring privacy and ethics contemplated by *Data Protection Law*. Make sure that blood extraction tubes are properly labeled and correspond to the available patient information.

TÍTULO: BLOOD PLASMA COLLECTION, PROCESSING AND STORAGE**ARCHIVADO:**

PNT_8.5.001_v1.1_EN_PLASMA_COLLECTION_PROCESSING_STORAGE

Nº DE CÓDIGO: 8.5.001

2. Label one biochemistry tube with anticoagulant EDTA-K3E/K2E for blood extraction.

7.3. OBTAINING BLOOD PLASMA

1. Extract blood in an identified extraction tube for hematology with EDTA.
2. Immediately after extraction, invert softly the tube to ensure proper mixing with the anticoagulant EDTA-K3E/K2E.
3. Take the tube to the lab to start processing it, ensuring there are no more than 45 minute time lapse between extraction and processing. Follow the rules for safe transportation of biohazard material established in your center.
4. Prepare 4-5 cryovials for plasma storage and label them properly.
5. Centrifuge the blood tubes (with anticoagulant) at 1500xg for 15 minutes without brake in order to avoid mixing the components. Three fractions will form:
 - Upper fraction or supernatant, clear and transparent layer with a pale yellow color. It is the blood plasma that needs to be stored.
 - Second or intermediate fraction, thin and grey or white, corresponds to the buffy coat (nucleated blood cells, mainly leucocytes).
 - Third or lower fraction forms a dark red layer and corresponds to erythrocytes.
6. With a sterile Pasteur pipette, carefully aspirate, if it is possible with a unique aspiration, the supernatant/plasma (upper fraction) and aliquot 0,5 ml into each cryovial.
7. Store cryovials in cryostorage boxes and place them in a -80°C freezer.
8. Register location of the stored sample in the Pulmonary Biobank Consortium's software.

8. APPLICABLE REFERENCES, LEGISLATION AND GUIDELINES

1. Declaration of Helsinki. <http://ohsr.od.nih.gov/helsinki.php3>
<http://www.wma.net/e/policy/b3.htm>
2. Tri-Council Policy Statement; Ethical Conduct for Research Involving Humans; Medical Research Council of Canada; Natural Sciences and Engineering Council of Canada; Social Sciences and Humanities Research Council of Canada, August 1998.
<http://www.pre.ethics.gc.ca/english/policystatement/policystatement.cfm>
3. Human Tissue and Biological Samples for use in Research. Operational and Ethical Guidelines. Medical Research Council Ethics Series. http://www.mrc.ac.uk/pdf-tissue_guide_fin.pdf
4. Best Practices for Repositories I. Collection, Storage and Retrieval of Human Biological Materials for Research. International Society for Biological and Environmental Repositories (ISBER). <http://www.isber.org>
5. National Bioethics Advisory Commission: Research involving human biological materials: Ethical issues and policy guidance, Vol. I: Report and recommendations of

TÍTULO: BLOOD PLASMA COLLECTION, PROCESSING AND STORAGE**ARCHIVADO:**

PNT_8.5.001_v1.1_EN_PLASMA_COLLECTION_PROCESSING_STORAGE

Nº DE CÓDIGO: 8.5.001

the National Bioethics Advisory Committee. August 1999.

<http://bioethics.georgetown.edu/nbac/hbm.pdf>

6. US National Biospecimen Network Blueprint

http://www.ndoc.org/about_ndc/reports/NBN_comment.asp

7. Blood Collection: Routine Venipuncture and Specimen Handling.

<http://medlib.med.utah.edu/WebPath/TUTORIAL/PHLEB/PHLEB.html>

UNCONTROLLED COPY