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SOP
Collection, Processing and Storage of Pleural Fluid Samples

Blood Products Working Group

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INDEX

1. ABBREVIATIONS

- **EDTA:** Ethylenediaminetetraacetic acid
- **PF:** Pleural fluid
- **DMSO:** Dimethyl sulfoxide (CH3SOCH3)
- **FBS:** Fetal bovine serum

2. DEFINITIONS

- **Pleural Fluid:** Is the fluid existing between the layers of tissue that line the lungs (visceral pleura) and chest cavity (parietal pleura). Based on its characteristics it can be classified into transudate (plasma filtrate) and exudate (inflammatory origin, rich in proteins and cells).
- **Pleural puncture or thoracentesis:** Is a procedure to remove excess fluid from the space between the outer lining of the lungs (visceral pleura) and the chest wall (parietal pleura).
- **EDTA: Ethylenediaminetetraacetic acid.** Anticoagulant agent that blocks the blood coagulation cascade by attracting ionic calcium.
- **Heparin:** Anticoagulant agent that prolongs blood clotting time by activation of antithrombin III.
- **DMSO:** Is a colorless organic liquid containing Sulphur; it is used as an organic solvent. Cryopreservative agent. Is a low-molecular-weight and membrane-permeable compound. It acts by displacing the water in the cell, avoiding the formation of ice crystals during freezing.

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 PF 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 PF 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 complies with the effective legislation: Royal Decree 664/97, following "Packing Instruction 620 (IATA - ICAO 602)"*
- *Syringes and/or material required for collecting PF*
- *Sterile tube for collecting PF*
- *Gloves for protection during handling*
- *Sterile 1 milliliter Pasteur pipettes*
- *Sterile cryotubes (from 0.5 to 2 milliliters)*
- *Cryotube racks*
- *Cryo storage boxes*
- *Labels appropriate for the type of cryotubes*
- *Centrifuge with adapters suitable for the type of collection tubes used*
- *Printer for labeling samples*
- *Freezing medium (FBS+10% DMSO)*
- *-80º C ultra-low temperature freezer with temperature recording system and a temperature maintenance system in case of power failure (CO2 injection, internal Energy Management System (EMS), generator) and telephone alarm system*
- *Sample management software applicable to each center (Examples: BBUN application (Maxwell), Bio-e Bank application, etc.)*

6. DEVELOPMENT

6.1. PRIOR CONSIDERATIONS

- Choice of anticoagulant: If the whole PF sample is taken for a specific purpose, it is recommended to choose the type of anticoagulant depending on the type of study/analysis that is to be done with the derived samples
- Cell proliferation studies-leukocytes: Heparin
- DNA/RNA extraction: EDTA.

6.2. COLLECTION OF PLEURAL FLUID

6.2.1. This is done by thoracentesis, after the patient has signed the informed consent (either for a specific study and/or for a biobank). It is recommended to extract 10-20 ml PF.

6.2.2. PF collection is performed in tubes with the anticoagulant chosen.

6.2.3. After obtaining the sample, it can be kept in a refrigerator at 4ºC until processing and subsequent storage (up to 48h).

6.2.4. The perfectly labeled sample and the request are transported to the laboratory together with the informed consent, while following the safety guidelines established by each center for the transport of biological material. It is recommended that the time between extraction of the PF and freezing at -80ºC is not more than 2 hours after extraction.

6.3. RECEIPT OF THE SAMPLE IN THE LABORATORY

6.3.1. 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.

6.3.2. Label and record the sample according to the sample management procedure used by the center.

6.3.3. Fill in the minimum data sheet with the data necessary for proper storage of the sample. It is advised to take the maximum possible information concerning the sample at the time of extraction:

- Date and time of extraction

- Type of anticoagulant

- Suspected diagnosis

- Incidents not related to the protocol.

6.4. PROCESSING: COLLECTION OF PLEURAL FLUID AND STORAGE OF THE ALIQUOTS

6.4.1. PF processing consists of obtaining the supernatant and the cell pellet.

6.4.2. Upon receipt of the sample in the laboratory, it is centrifuged between 800-1200g for 10 minutes at 4ºC.

6.4.3. Carefully aspirate the clear and transparent yellowish supernatant, using a sterile Pasteur pipette or a micropipette of adequate volume.

6.4.4. The cell pellet is frozen in freezing medium (FBS+10% DMSO) at -80ºC in suitable cryogenic vials.

6.4.5. Divide the supernatant into aliquots of 0.5-1ml ml in suitable cryogenic vials that are properly labeled and identified.

6.4.6. Close the tubes properly to obtain an airtight seal. Record the number of aliquots obtained for each sample. Store at -80ºC.

6.4.7. Record the location of the sample in the sample management software used by the biobank.

7. REFERENCE DOCUMENTATION

- Standard ISO 9001:2008. Quality management systems. Requirements.
- Organic Law 15/1999 of 13 December on the Protection of Personal Data (LOPD).
- Law 14/2007, of 3 July, on Biomedical Research (LIB).

8. RELATED DOCUMENTATION

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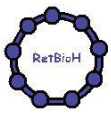

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