TÍTULO: FLASH FROZEN PRESERVATION OF LUNG TISSUE		
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1. OBJECTIVE

Tissue samples are collected from surgical surplus from patients that have signed the informed consent form and agree with the participation in the Pulmonary Biobank Consortium.
Fresh frozen tissue collections are a valuable resource for research. Tissues samples are only suitable for proteomic and genomic studies if they have been properly frozen. This document aims to normalize the flash frozen protocol for the Pulmonary Biobank Consortium.

2. SCOPE

This protocol describes how to quickly freeze lung tissue. This procedure does not detail biohazard and/or chemicals processes for occupational safety and health. It is recommended that the staff follows the established Health and Safety rules of each center.

3. RELATED DOCUMENTS

PNT_8.2.001_Recogida y transporte Tejido Pulmonar.doc
PNT_8.1.001_Etiquetado y Trazabilidad.doc

4. ROLES AND RESPONSIBILITIES

The fulfillment of this standard protocol rests with all the members of the Pulmonary Biobank Consortium who are responsible for the flash freezing of fresh lung tissue.

5. MATERIALS AND EQUIPMENT

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

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Materials and equipment	Materials and equipment (Specific to Center)
Containers for liquid nitrogen transportation	
Liquid nitrogen	
2-methylbutane (isopentane)	
Isopentane container (bath in liquid nitrogen or dry ice)	Histobath or equivalent
50ml tubes (or 10 ml minimum)	
Liquid nitrogen resistant 1,5-2ml cryotubes	
Filter paper	
Suitable containers for cut tissue (Petri dishes)	
Pens and Markers	
Sterile forceps	
Cooled (4ºC) PBS or saline solution tissue rinsing	
Scissors to cut tissue	
Rack for cryotubes and 50ml (or 10ml) tubes	
Gloves to protect staff from tissue handling	
Adequate and suitable labels for collection tubes	
Splash protection mask	
Lab coat for protection against spills and splashes	
Protection mask suitable for liquid nitrogen handling	
Suitable gloves for liquid nitrogen handling	

6. GLOSSARY

Cryopreservation: Biological conservation process that consists on the preservation at very low temperatures for a long period of time.

7. PROCEDURE

The objective of this procedure is to guarantee safety, timely and efficient tissue sample preservation from patients that have signed the informed consent form and agree with the participation in the Pulmonary Biobank Consortium. Thereby this protocol tries to avoid contamination risk and molecular integrity loss. The sample preservation process is essential for high integrity and quality products obtainment, which facilitate the use of genomic and proteomic techniques.

7.1 TISSUE TRANSPORT AND FLASH FREEZING OF LUNG TISSUE

1. Treat the tissue as potentially infectious.
2. Freezing is carried out by the laboratory technician or the person designated by the head of each Pulmonary Biobank Consortium node.

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3. Identify and/or label cryotubes and prepare all the material before the call from operating room.
4. If immediate freezing is not possible, tissue should be processed within the first 30 minutes after resection. To ensure it, manual or digital registration of time should be performed.
5. If time between resection and the beginning of tissue conservation is over 30 minutes, it should be indicated as “incidence” in the sample information field or in the “observations” field. If time exceeds 40 minutes, the tissue collection according to this protocol should not be done.
6. Do not place the tissue directly on ice.
7. Ensure that the tissue sample does not dry out or become contaminated by contact with other tissues or samples. Use clean and sterile dissection material. Avoid cross contamination between samples or between tumoral and healthy regions.
8. Directly frozen tissue is suitable for DNA, RNA and protein extraction. Avoid formalin exposure of the sample in any step of the process. Do not add serum to the sample.
9. Place the sample in PBS or saline solution pre-cooled at 4ºC in a 50ml sterile container.
10. Wash 3 times in pre-cooled PBS or saline solution, shaking for 3-4 minutes or static for 10 minutes.
11. Lightly dry the sample on filter paper.
12. Cut the sample in 0,3 x 0,3 x 0,3 cm fragments with the aid of sterile scissors.
13. Cool down isopentane holding its container over liquid nitrogen or dry ice. Isopentane is cool enough when it appears like pearls and the solution gets a white and dense appearance.
14. With the aid of sterile forceps place 3 fragments sufficiently separated on the inner wall of a criotube. Use 1,5-2ml cryotubes with screw cap.

    NOTE: If there are more than one fragment per cryotube, make sure they do not clump at the bottom of the tube.
15. Close the cryotubes and submerge them in cool isopentane until freezing (it should take 30 seconds).
16. Alternatively to isopentane freezing, cryotubes can be frozen submerging them immediately in liquid nitrogen. Samples should be frozen in 30-60 seconds. This alternative step is not recommended for big samples because it is necessary more time and loss of morphology could happen.
17. Once frozen, transfer the sample to a -80ºC freezer. Use liquid nitrogen or dry ice for transportation.
18. Register location of the stored sample in the Pulmonary Biobank Consortium’s software.

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8. APPLICABLE REFERENCES, LEGISLATION AND GUIDELINES

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