TÍTULO: LUNG TISSUE PRESERVATION IN PARAFORMALDEHYDE AND OCT		
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1. OBJETIVE

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.

Paraformaldehyde-fixed, OCT-embedded (*Optimal Cutting Temperature*) lung tissue collections are a valuable resource for research. Paraformaldehyde is one of the most extended fixation agents since it preserves a wide range of tissues and tissue components, preserving ultra-cellular structures (histology). This method is effective to preserve histological morphology of lung tissue. This document aims to normalize and standardize the paraformaldehyde-fixed, OCT-embedded protocol for lung tissue preservation for the Pulmonary Biobank Consortium.

2. SCOPE

This protocol describes how to preserve lung tissue with paraformaldehyde and OCT. 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 fulfilment of this standard protocol rests with all the members of the Pulmonary Biobank Consortium who are responsible for preserving the lung tissue in paraformaldehyde and OCT.

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)
Containers for liquid nitrogen transportation	
Liquid nitrogen	
2-methylbutane (isopentane)	
Isopentane container (bath in liquid nitrogen or dry ice)	Histobath or equivalent
Suitable containers for handling and cutting (Petri dishes)	
Pens and Markers	
Sterile forceps	
PBS or saline solution	
Vinyl cryomolds (such as Tissue-Tek Ref.#4557 25mm x 20 mm x 5 mm)	
4% paraformaldehyde (o Polyoxymethylene) in PBS	
Freezing medium OCT (Tissue-Tek O.C.T. Ref.#4583)	
Scissors to cut tissue	
Gloves to protect staff from tissue handling	
Adequate and suitable labels for cryo molds	
Splash protection mask	
Lab coat for protection against spills and splashes	
Protection mask suitable for liquid nitrogen handling	
Gloves suitable 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.

OCT: "*Optimal Cutting Temperature*" refers to a compound used to preserve tissues against freezing. OCT preserves ultra-structures and protects the tissue from dehydration and degradation. It acts as an isolator on temperature variations and minimizes crystal formation. It is especially useful for preservation of frozen fresh tissue that will be sectioned.

PARAFORMALDEHYDE: Polyoxymethylene is used as fixative agent for cells in histology. It is a product of polymerization of formaldehyde (8-100 units).

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 TRANSPORTATION

1. Tissue mobilization must be quick; no more than 30 minutes between resection and the beginning of tissue conservation. To ensure it, manual or digital registration of time should be performed.
2. 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.
3. The operating room staff will notify the tissue availability to the person responsible of tissue obtainment who will organize the tissue transport from the operating room to the assigned lab. Transportation will be organized optimally to preserve cellular and molecular integrity.
4. The pathologist or responsible delegated person will determine the tissue fragment surplus for biobank based on clinical needs.
5. Tissue sample must be introduced into a plastic container, which in turn is inside a container filled with ice.
6. If possible, prepare the tissue collection kits previously.

7.2 LUNG TISSUE FIXATION IN 4% PARAFORMALDEHYDE PBS

1. Treat the tissue as potentially infectious and do not place it directly on ice.
2. Fixation and freezing is carried out by the laboratory technician or the person designated by the head of each Pulmonary Biobank Consortium node.
3. Unless it can be fixed immediately, the tissue should be processed within 30 minutes after resection.
4. Make sure that sectioned tissue does not dry out or become contaminated by contact with other tissues or samples. Use always clean and sterile forceps and scissors. Avoid cross contamination between samples or between tumoral and healthy regions.
5. Place a 1 x 1 x 0,2 cm fragment in a 50ml container with pre-cooled (4ºC) PBS or saline solution.
6. Wash the sample 3 times with pre-cooled saline solution shaking for 3-4 minutes or static for 10 minutes

7. Place the sample in 5-10ml of pre-cooled 4% paraformaldehyde in PBS. Incubate at 4ºC for 24h.
8. Cut approximately 0,3 x 0,3 x 0,2 cm pieces with the aid of sterile scissors (to prevent tearing) and place them in a sterile container with OCT:PBS (1:3).
9. Incubate at room temperature for 1h.
10. Put the samples in a new container with OCT:PBS (1:1) solution.
11. Incubate at room temperature for 1h.
12. Put the samples in a new container with OCT solution and incubate at room temperature for 1h.

7.3 OCT-FIXED LUNG TISSUE FREEZING

1. 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.
2. Place some drops of OCT medium in the cryomold in order to make a thin layer of OCT. Cryomolds must be previously labeled and/or identified.
3. With sterile forceps, place 1 fragment into the cryomold with OCT. An adequate sample orientation in the cryomold is important to obtain optimal histological cuts in the cryostat.
4. Cover the lung tissue fragment with OCT.
5. Use forceps or a pipette tip to place the tissue properly oriented and remove any air bubbles.
6. Place the cryomold into pre-cooled (-50ºC) isopentane (in histobath or liquid nitrogen bath) with the aid of forceps. Avoid contact between isopentane and the OCT, to prevent air bubbles and lung tissue tearing.
7. Transfer the frozen cryomold to a -80ºC freezer. Use liquid nitrogen or dry ice for transportation.
8. Register the stored sample location in the Pulmonary Biobank Consortium's software.

Protocols for tissue preservation are available in PNT 8.2.002, 8.2.003, 8.2.004, 8.2.005 y 8.2.006.

8. APPLICABLE REFERENCES, LEGISLATION AND GUIDELINES

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