TÍTULO: LUNG TISSUE PRESERVATION AND INCLUSION IN FORMALIN-PARAFFIN		
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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. Part of the samples is preserved in paraffin blocks. Histological sections can be obtained from paraffin blocks using a microtome.

Formalin-fixed, paraffin-embedded (FFPE) lung tissue collections are a valuable resource for research. They are easily stored at room temperature for long periods of time. Formalin is the most extended fixation agent since it preserves a wide range of tissues and tissue components. This method is effective to preserve histological morphology of lung tissue. This document aims to normalize and standardize the lung tissue preservation in formalin-paraffin protocol for the Pulmonary Biobank Consortium.

2. SCOPE

This protocol describes how to preserve lung tissue with the FFPE method. This procedure does not detail the processes for occupational safety and health regarding biohazard material and/or chemicals, and it is recommended that the staff follows the established Health and Safety rules of each centre.

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 with the FFPE method.

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.

Materials and equipment	Materials and equipment (Specific to Center)
PBS or saline solution	
Formaldehyde (or methylaldehyde; H_2CO) 3,5-4% pH7	
50 ml polypropylene tube	
Suitable containers for handling and cutting (Petri dishes)	
Pens and markers	
Graphite pencil or Cassette Printing System	
Sterile forceps	
Filter paper	
Scissors to cut tissue	
Gloves to protect staff from tissue handling	
Adequate and suitable labels for cryomold	
Splash protection mask	
Lab coat for protection against spills and splashes	
Histology cassettes (paraffin inclusión)	
Histology cassettes caps	
Temperature-resistant gloves for personal protection	
safety cabinet for gas extraction	
Alcohol (Ethanol)	
Xylene or substitute	
Paraffin embedding station	
Paraffin molds	
Weight rod for paraffin block processing	
Paraffin (low melting point)	

6. GLOSSARY

Preservation: Procedure to avoid or delay the biological or physical deterioration of the sample using chemicals, altering environment conditions or other actions.

Dehydration: Water extraction from tissue.

7. PROCEDURE

Formalin fixation is a standardized protocol in histopathology labs. The following steps allow proper tissue preservation in formalin:

1. Tissue samples should not be bigger than 1.5 x 1 x 0.5cm.
2. A fixation defect has a higher risk in the quality process, but an excess of fixation can also carry problems when applying immunohistochemical methods.

It is advisable that the lab is equipped with an automatic processor for paraffin embedding with standardized processing times. However, the following protocol can be used as a reference guide.

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, a 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 60 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 which tissue fragment surplus goes to 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 PRESERVATION IN FORMALIN:

1. Preservation is carried out by the laboratory technician or someone trained to do so, designated by the head of each Pulmonary Biobank Consortium node. It should be done as soon as possible after resection.
2. Treat the tissue as potentially infectious.
3. Identify and/or label one polypropylene tube (50 ml) and prepare all the material before the call from operating room.
4. The tissue must be placed in the polypropylene tube with approximately 50 ml of saline solution pre-cooled at 4ºC, incubate on ice.

5. Wash the sample 3 times with pre-cooled saline solution shaking for 3-4 minutes or static for 10 minutes.
6. Cut 0,5 x 0,5 x 0,2 cm pieces with the aid of sterile scissors (to prevent tearing). Depending on tissue availability, the size may vary from 0,2 x 0,2 x 0,2 to 1 x 1 x 0,2 cm.
7. Place each tissue piece in an appropriated cassette and place its cover. Identify cassettes according to each center or pathology lab with a graphite pencil or a cassette printing system.
8. Place the pieces in 3,5-4% formalin solution (pH 7) in a minimum proportion of 20:1 (fixative:tissue).
9. Incubate the samples at room temperature (25ºC) for at least 24h and a maximum of 72h.

NOTE: The following steps are standardized in every center. They can be used as a reference guideline, but there might be minor differences between centers.

7.4. DEHYDRATION AND RINSING PROCESS

1. Dehydrate tissues in a graded series of alcohols (70%, 96% y 100%).
2. Rinse the lung tissue in xylene or xylene substitute (Citrus).
3. Dehydrate and rinse the tissue following these steps:

STEP	TIME	SOLUTION
1	60min	Ethanol 70%
2	60min	Ethanol 96%
3	60min	Ethanol 96%
4	60min	Ethanol 100%
5	60min	Ethanol 100%
6	60min	Ethanol 100%
7	60min	Xylene (or xylene substitute)
8	60min	Xylene (or xylene substitute)

4. After completing the dehydration and rinsing processes, place the cassettes with the pieces in hot paraffin (approximately 58ºC) until starting the paraffin inclusion process.

7.5. PARAFFIN INCLUSION

1. Use preferably low melting point paraffin supplemented with DMSO to ensure the quality of nucleic acids. Ensure that the paraffin has reached 65ºC temperature by

acquiring a liquid state, thereby allowing the tissue inclusion and subsequent block formation.

2. Choose a suitable mold according to the sample. Fill the mold base with liquid paraffin and place tissue on it. Guide the tissue leaving the flat and wide part at the mold bottom, allowing an homogeneous cutting.
3. In the paraffin embedding hot zone, crush the sample with the weight rod using only vertical force, do not rotate the rod or apply shearing forces (to not tear sample structure). Lift the rod in order to let the air bubles escape.
4. Push once again the sample with the rod and transfer the mold with the tweezers to the cold zone (3 seconds). Wait to see a forming layer of solid paraffin in the bottom of the mold, quickly lift the rod and fill the mold with hot paraffin. Transfer again the mold to the cold zone and insert the identified histology base cassette on top of the mold. Fill the mold once again with melted paraffin and transfer it to the coold down station for about 15 minutes.
5. Unmold the paraffin cassettes (blocks). With the aid of a knife or scraper remove excess paraffin from block borders.
6. Store paraffin cassettes at room temperature (25ºC) or lower. Prevent exposure to sun or to extreme temperature variation. Store cassettes in storage boxes properly labeled and/or identified, adding the Pulmonary Biobank Consortium label.
7. Register location of the stored sample in the Pulmonary Biobank Consortium's software.

8. APPLICABLE REFERENCES, REGULATION AND GUIDELINES

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