TÍTULO: LUNG TISSUE PRESERVATION IN RNALATER		
ARCHIVADO: PNT_8.2.002_v1.4_EN_LUNG_TISSUE_RNA_LATER		**Nº DE CÓDIGO:** 8.2.002
VERSIÓN: 1.4	**FECHA DE EDICIÓN:** 25/07/2017 09:07:00 O7/P7	**PÁG.** 1 DE 4

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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.

Lung tissue collections preserved in RNA*later*® solution are a valuable resource for research. Tissues preserved in RNA*later*® are mainly used for studies that need excellent RNA quality. Furthermore, they can be used for proteomic and genomic studies. This document aims to normalize and standardize lung tissue preservation in RNA*later*® protocol for the Pulmonary Biobank Consortium.

2. SCOPE

This protocol describes how to preserve lung tissue in RNA*later*® solution. 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 RESPONSABILITIES

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 RNAlater® solution.

5. MATERIALS AND EQUIPMENT

Recommended materials and equipment are listed below are. 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)
Suitable containers for handling and cutting (Petri dishes)	
Pens and Markers	
Sterile forceps	
RNAlater® solution	
Dissecting Scissors (sterile)	
Gloves to protect staff from tissue handling	
Adequate and suitable labels for cryotubes	
Splash protection mask	
Lab coat for protection against spills and splashes	
Scotch tape	
Sterile cryotubes	
Pipette 1000ml and sterile tips	

6. GLOSSARY

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

RNA*later*® Solution: RNA*later*® solution is an aqueous, nontoxic, tissue storage reagent that rapidly permeates most tissues to stabilize and protect cellular RNA. RNA*later*® solution minimizes the need to immediately process fresh tissue samples or to freeze samples in liquid nitrogen for later processing. RNAlater treated samples can be stored at -20°C (or lower) indefinitely. It is especially useful for the preservation of RNA quality and quantity.

7. PROCEDURES

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

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 PRESERVATION IN RNA*later*® SOLUTION:

1. Handle the tissue as potentially infectious.
2. Do not place the tissue directly on ice.
3. Preservation is carried out by the laboratory technician or the person designated by the head of each Pulmonary Biobank Consortium node.
4. Identify and/or label cryotubes and prepare all the material before the call from operating room.
5. Unless it can be placed in the RNAlater® solution immediately, the tissue should be preserved within 30 minutes after resection.
6. 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.
7. Avoid sample contact with formalin in any step of the process. Do not add serum to the sample.
8. Place a piece of tissue in a small flat container (e.g. a 50ml falcon tube lid or a urine container’s lid) with approximately 5ml of RNA*later*® solution to let it permeate the sample while tissue handling.
9. Using scissors (to prevent tearing) and sterile forceps, cut slice pieces of 0,2 X 0,2 X 0,2 cm.

10. Place 3-10 fragments in each cryotube with 0,5 ml of RNA*later*® solution.
11. Incubate the samples 24h at 4ºC.
12. Store the samples at -80ºC or lower (if lower, preserve the minimum amount of tissue in each cryotube to send to the applicant researcher, avoiding manipulation sample once frozen).
13. Register location of the stored sample 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, REGULATION AND GUIDELINES

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