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OSU TriState SenNet Processing and Storing of Normal Donor Lungs

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TriState SenNet | Cellular Senescence Net…

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We use this protocol and it's working

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Abstract

This protocol describes the processing and storing of normal donor lungs by the Comprehensive Transplant Center (CTC) Human Tissue Biorepository before arrival at the TriState SenNet Biospecimen Core of The Ohio State University that functions as a part of the Cellular Senescence Network Program (SenNet). This protocol is based on the CTC Human Tissue Biorepository's standard operating procedure established for tissue collection for explant and donor samples. We follow routine laboratory specimen processing guidelines to ensure the highest biospecimen quality.

Materials

·Nitrile gloves
·Disposable gown
·Biosafety cabinet (BSC)
·Underpads (blue absorbent pads)
·Large and small biohazard bags
·Small biohazard bag holder, used to hold the bag in BSC for ease and efficiency
·Nalgene biohazard bin lined with red medium biohazard bag
·Autoclaved surgical kit containing 2 surgical scissors, 2 tweezers, and 2 hemostats
·Cryovial rack
·5 mL cryovials
·2 mL cryovials
·10% neutral buffered formalin (NBF)
·20 mL glass scintillation vial
·70% ethanol
·Disposable 11 blade scalpel
·Tissue cassettes
·Optimal cutting temperature compound (OCT)
·Hydroxypropyl methylcellulose (HPMC)
·polyvinylpyrrolidone (PVP)
·mold

Objective

1 To preserve lung tissue for further downstream cellular, protein, RNA, or DNA analyses.

Preparation

2

2.1 In the biosafety cell culture hood, place three underpads and all needed equipment, including biohazard receptacles, surgical kits, and specimen holders. Before beginning, check that all appropriate PPE is donned and BSC airflow is on before processing samples.

2.2 Lungs come triple-bagged in HTK solution or saline from our local organ procurement organization (Lifeline of Ohio). Place the container holding the specimen inside the BSC, remove lungs from containers, place them on the underpad, and photograph before sampling. Be sure that the photograph shows both lungs with their respective lobes, front and back.

2.3 For normal donor samples, the lung tissue is spongy. Healthy tissue feels soft and elastic, and the appearance is pink. Carbon deposits are common in all human lung samples and sometimes cannot be avoided. When processing normal donor tissue, look for the healthiest region to sample. The donor's CT or chest x-ray can provide information to select the best locations. Select the sites from which samples will be collected and annotate in the notebook.

2.4 From the upper and lower of the same lung, remove a 10 cm^3 area piece of tissue. The height will vary for each lung specimen.

2.5 Collect four pulmonary lymph nodes near or around the primary bronchus (hilar region) in a 5mL cryovial.

Freezing and storing

3

3.1 Cut six small pieces of tissue (4mm^3) from each piece of parenchyma and place them onto the internal sides of a labeled cryovial using forceps. Collect ten cryovials of parenchyma from the upper, lower, or both lobes.

3.2 Lower the cryovials into liquid nitrogen and leave for 5 minutes.

3.3 After freezing, remove the cryovials from liquid nitrogen and store them in a -80°C freezer.

Storing for FFPE and OCT processing

4 **1. For FFPE:**

a. Add 3mL 10% neutral buffered formalin (NBF) into a 5mL cryovial containing normal donor lymph nodes.

b. Add 10 mL 10% NBF into a labeled 20 mL glass scintillation vial, and place four 1.25 cm^3 pieces of tissue from normal donor lung parenchyma into the vial.

c. Top off the vial with NBF if needed and protect it from light. Samples must be submerged for at least 24 hours and no longer than 72 hours to ensure complete saturation and to prevent over-fixation.

d. Remove tissue from NBF and use a scalpel to cut the tissue into a <2mm thick piece that is small enough to fit into the tissue cassette.

e. Enclose tissue into the cassette and then submerge the cassette in 70% ethanol.

f. Embedding and histology are performed using standard methods.

2. For OCT:

a. Cut the tissue into 1 x 1 cm pieces for freezer preservation in OCT at -80°C.

b. Transfer the piece of tissue into 4% paraformaldehyde at 4°C for 24 h.

c. Transfer to 30% sucrose solution at 4°C for 24 h, then embed in a mold with OCT.

d. Store in a -80°C freezer.

3. For Lipidomics and Proteomics analyses:

a. Cut the tissue into 1 x 1 cm pieces for freezer preservation in 75% of Hydroxypropyl methylcellulose (HPMC) and 2.5% of polyvinylpyrrolidone (PVP) at -80°C.

b. Embed in a mold with HPMC-PVP.

c. Store in a -80°C freezer.