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OSU TriState SenNet Processing and Storing of Explanted IPF Lungs

Forked from OSU TriState SenNet Processing and Storing of Explanted IPF 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 explanted idiopathic pulmonary fibrosis (IPF) 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 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 Use appropriate PPE that includes nitrile gloves and disposable gown or washable lab coat.

3 Place three underpads and all needed equipment, including biohazard, receptacles, surgical kit, and specimen holders in the biosafety cabinet (BSC) and turn on.

4 Lungs come from the OR separated left from right lungs in sterile plastic containers. Place container inside the BSC, remove lungs from containers, place on the underpad, and photograph lungs prior to sampling. The photograph should show both lungs with their respective lobes, front and back.

5 Samples are collected from the area most representative to the disease. IPF lung tissue is fibrotic and this is characterized by the thickening and scarring of the tissue that feels hard and stiff to the touch. Identify the most representative areas in the upper and lower lobes of a lung.

6 Resect a 10 cm x 10 cm x 2 cm (L x W x H) piece of parenchymal tissue from the representative sections of the upper and lower lobes and set aside.

7 Collect four pulmonary lymph nodes around the primary bronchus in 5mL cryovials.

Freezing and storing

8 The following procedure is performed for the upper lobe and the lower lobe.

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

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

11 Remove the cryovials from liquid nitrogen and store in the -80°C freezer.

Processing formalin fixed paraffin embedded (FFPE) and OCT samples

12 The following procedure is performed for the upper lobe and the lower lobe.

13 **For FFPE preserved samples:**

13.1 Add 3mL 10% neutral buffered formalin (NBF) to the 5mL cryovial containing lymph nodes

13.2 Cut four 1.25 cm^3 pieces of tissue from each piece of parenchyma and place into a labeled 20 mL glass scintillation vial filled halfway with NBF. Top off NBF if needed and protect from light. Samples must be submerged for at least 24 hours and no longer than 72 hours to ensure complete saturation and to prevent overfixation.

13.3 Remove tissue from NBF and use a scalpel to cut the tissue into <2mm thickness pieces that are small enough to fit into the tissue cassette.

13.4 Enclose tissue into the cassettes and then submerge in 70% ethanol. Histology is performed using standard methods.

14 **For OCT preserved samples:**

14.1 Cut tissue into 1.25 cm^3 pieces and transfer tissue into 4% paraformaldehyde for 24 hours.

14.2 Transfer to 30% of sucrose for 24 hours and then embed in a mold with OCT and dry ice.

14.3 Store in -80°C freezer.

15 **For Lipidomics and Proteomics analyses:**

15.1 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.

15.2 Embed in a mold with HPMC-PVP.

15.3 Store in a -80°C freezer.