ABSTRACT

Purpose and Scope of the Procedure

1. Standardize process for processing lung donations into components for storage and distribution
2. Scope: coordination of receipt and gross dissection of tissue

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GUIDELINES

Scientific Principles

1. Rapid, standardized and safe processing of human tissue for the HTC requires coordination of a team of staff, materials and attention to protocols
2. Rapid processing is required to maintain the tissues in a state as close to normal as possible
3. Ultra-High Resolution CT Scan in an inflated state will provide a high-level comparative assessment of human lung structure across developmental ages

MATERIALS TEXT

MATERIALS

10X PBS; Diluted to 1X with DEPC H2O

Contributed by

users Catalog #Corning 46-013-CM
20% Paraformaldehyde (PFA); Diluted to 4% (m/v) PFA in 1X PBS Contributed by

10% Buffered Formalin Contributed by

Diethylpyrocarbonate (DEPC) Contributed by

Ethanol Contributed by

OCT (inflation); 50% (v/v) OCT in 1X PBS Contributed by

OCT (mold) Contributed by

Sucrose; 30% (m/v) Sucrose in 1X PBS Contributed by

Carboxymethylcellulose (CMC); 5% (w/w) CMC in DEPC H2O Contributed by

Worksheet

Grossing Station/ Biosafety Cabinet or Fume hood
Gloves, face protection and clothing consistent with blood and body fluid precautions
Heavy Duty Scissors and Wire Cutters
Biohazards Disposal Bag
Red Sharps Container
Biosafe surface for manipulation of lung
Small and Medium Gauze Pads – multi-pack
Standard Balance and Weigh Boats: Medium and Large
Airway Cannulas – Selection of sizes
16 and 18 gauge angioplastics (needles removed and discarded)
Tracheal cannulas 2.0-4.0 OD
Endotracheal tubes 2.5 – 8 mm OD; tubes >/= 4.0 cuffed
IV extension set tubing with clamp
20 ml syringe barrels for fixative
Needle free suture material
Zip Ties
Dissection Instruments: Same or similar to Sakura Series:
#4791 Scalpel Handle with
#4792 or #4793 Scalpel Blades, #61 (curved tip) or #62 (pointed tip), resp
#4785 Trimming Blades, Short and/or #4789 Trimming Blades, Long
#4786 Trimming Handle, Short and/or #4790 Long Straight
#4794 Blade Scissors with #4796 Blades Sharp/Blunt
#4807 Accu-Edge® Grossing Fork 2.5 mm
#4800 Accu-Edge® Grossing Board Inches (L x W x D): 17 x 11.5 x 1
#4802 Accu-Edge® Grossing Wells Inches (L x W x D): 12.9 x 3.25 x 2.15
#8031 Slide Printer
a. #8033 Slide Unload Station
b. #8035 Ink Cartridge
c. #8040 Slide Magazine
Mopec Product #AB079 ProCut Forcep Kit
Camera – iPad in water proof case works well
10% neutral buffered formalin – at least one liter with more on hand if needed for larger lungs
1X Phosphate Buffered Sodium (PBS diluted in DEPC treated water)
Paraformaldehyde (prepared from 1:4 dilution of 16% stock non-buffered, no menthol formalin in 1x PBS in DEPC water)
Liquid Nitrogen in Dewar flask

Citation: Gloria S Pryhuber, Heidie Huyck (08/16/2020). 603.3 & 604.5 URMC_HTC_Whole Lung and Lobe Processing.
https://dx.doi.org/10.17504/protocols.io.biz7kf9n

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SAFETY WARNINGS
Personnel will adhere to safe work processes outlined in U.S. Public Health Universal Precautions Guidelines for use of human blood and body fluids. PPE will be used including lab coat, closed shoes and gloves. All activity will be behind shield of biosafety cabinet and/or a grossing station with mask and safety glasses. Biosafety level 2 practices will be followed and the work performed in the designated lab space that is covered by annually updated IBC approved protocol. All institutional biosafety measures are followed in any manipulation of these human tissues.

1. Assemble Team and Materials as needed for processing, to accomplish:
   i. CT Scan
   ii. Separation of Lobes
   iii. Tissue Dissociation
   iv. Formalin Inflation
   v. OCT/CMC Inflation
   vi. Paraformaldehyde Inflation

2. Record details of procedures in Worksheets.

Unpacking

3. Shipped package is opened on arrival to remove a blood sample, if provided, as it should be kept at room temperature. The remainder of the package is to be kept in cold room until processing begins to maintain approximately 4 °C temperature of tissue.

4. Determine BRINDL PID to assign to tissues and images by clicking the "arrived" button in BRINDL Screening log

5. Record Information on Shipping Labels in Database or worksheet for entry ASAP
   i. UNOS #; Referring Company #
   ii. Courier and Tracking Numbers

6. Remove Shipping Labels from package; place aside to be added to Sample File

7. Open Shipping Box and Remove Styrofoam Cover
   i. Note Condition of Packing in Database (ice sufficient, layered packaging, no leakage, etc.)

8. Open container containing tissue and move lung tissue to a clean plastic bag on ice.

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This is an open access protocol distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
i. Note Condition of Lung Tissue in Database
   a. Is tissue submerged (usually in plastic bag, inside a hard plastic container)
   b. Are Trachea and both lungs included and intact
   c. Is Trachea Occluded, if so how
   d. Are Thymus, Spleen, Blood Sample, Lymph Nodes included?

CT Scan
9  See separate protocol for CT Scan of Air Inflated Lung procedures.

Grossing of Tissues
10  Maintain tissues in transplant buffer on ice. Prevent open Trachea from being submerged, +/- ETT removal

11  Weigh intact organ as sent; Continue to Record all measurements and procedures in Worksheet or directly in BRINDL database

12  Determine displacement volume without inflation

13  Determine plan for organ lobes ie which to be inflated or infused and which to be dissociated

14  Remove excess tissues around trachea and large airways at hilum
    Collect lymph nodes, excess large vessels, esophagus and nerves
    (Best to place these back in storage buffer and Tend to Lung First)
    a.  Weigh each tissue collected
    b.  The non-lung samples are sectioned into approximately 0.5-1 cm3 portions
    c.  Divide these tissues between
        i.  10% formalin, place in labeled tissue cassette in formalin
           Store at \(\delta 4^\circ C\) to fix for 44-48 hrs,
           Section at mid-time.
           Volume of fixative to tissue should be approximately 10:1.
        ii. PFA: place in 4%PFA stored at \(\delta 4^\circ C\) to fix for 20-28 hours before moving to 30%Sucrose
    d.  Record these tissues and how processed in BRINDL database

15  Dissect free the bronchial branches, identify pulmonary arteries and veins

16  Divide airways and vessels to obtain 5 independent lung lobes

16.1  Weigh each lobe once isolated
16.2 Proceed with processing for each lobe as outlined in Table 1 using appropriate Protocols.

17. Prepare accompanying tissue such as thymus, spleen, esophagus, blood, heart, in similar fashion with specific as outlined in the appropriate Protocol / SOP.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Starting Tissue</th>
<th>Prep</th>
<th>Process</th>
<th>BioSpecimen Type Collected from Organ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trachea and large bronchi</td>
<td>Intact</td>
<td>Paraffin and OCT Frozen</td>
<td>Bisect longitudinally; Section into &lt;1 cm long half rings</td>
<td>Formalin Fixed, Paraffin embedded PFASOCT embedded and frozen</td>
</tr>
<tr>
<td>Alternate Trachea and large bronchi</td>
<td>Intact</td>
<td>Cell Isolates</td>
<td>Enzymatic digestion and cell isolation</td>
<td>Frozen isolated cell aliquots</td>
</tr>
<tr>
<td>Lung: Right Upper Lobe +/- Right Middle Lobe</td>
<td>Fresh Tissue Blocks</td>
<td>Mixed Dissociated Cells</td>
<td>Dissociate cells by mechanical and enzymatic digestion procedure</td>
<td>Cryopreserve mixed cell aliquots</td>
</tr>
<tr>
<td>Sorted Dissociated Cells</td>
<td>Flow Activated Cell Sorting dissociated cells</td>
<td>Cryopreserve sorted cell aliquots “ex. Endothelial, Epithelial, Fibroblast, Bone Marrow Derived (CD45+) Separation”</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryo-preserved QI</td>
<td>Cryo-preserved cells</td>
<td>Recover frozen cell aliquots, test purity by PCR, culture for viability and proliferative/differentiation potential</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Add'l Right Middle Lobe</td>
<td>Intact - Biopsies</td>
<td>Flash Frozen</td>
<td>Stored at -80</td>
<td>Protein Homogenates, RNA and/or DNA Isolation</td>
</tr>
<tr>
<td>Alternate Right Middle Lobe</td>
<td>Intact</td>
<td>Formalin Inflation, Paraffin</td>
<td>Inflation fixed with 10% buffered formalin x 24 hr (Intact Lobe); Sectioned by grid into 0.5-1 cm3 pieces; Dehydrated to 70% Ethanol</td>
<td>Inflation fixed, paraffin embedded ready for sectioning</td>
</tr>
<tr>
<td>Lobe</td>
<td>Sectioning Method</td>
<td>Embedding Material</td>
<td>Embedding Method</td>
<td>Notes</td>
</tr>
<tr>
<td>--------------------------</td>
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<td>----------------------------</td>
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<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>Alternate Right Middle Lobe</td>
<td>Fresh Tissue Blocks</td>
<td>Fresh Frozen Embedded in OCT/CMC/none</td>
<td>Vertical 0.1 cm slices, Section each slice by grid into 1 cm2 blocks, alternate slices to embed blocks in OCT or approx. 5% CMC (see protocol) or freeze without embedding material</td>
<td>Non-inflated, non-fixed flash frozen tissue blocks, mapped to originating location</td>
</tr>
<tr>
<td>Right Lower Lobe</td>
<td>Intact</td>
<td>Formalin Inflation, Paraffin</td>
<td>Inflation fix with 10% buffered formalin x 24 hr (Intact Lobe) Sectioned by grid into 0.5-1 cm3 pieces Dehydrated to 70% ethanol</td>
<td>Inflation fixed, paraffin embedded ready for sectioning</td>
</tr>
<tr>
<td>Left Upper Lobe</td>
<td>Intact</td>
<td>PFA Fixed, Sucrose Cryopreserved, OCT embedded</td>
<td>Inflation fixed with 4% paraformaldehyde x 24 hr (Intact Lobe); Sectioned by grid into 0.5-1 cm3 pieces; Cryopreserved in 10% sucrose; Block in OCT</td>
<td>Inflation fixed, cryo-preserved and frozen for cryo-sectioning</td>
</tr>
<tr>
<td>Alternate Left Upper Lobe</td>
<td>Intact</td>
<td>OCT/PBS</td>
<td>Inflate with 50:50 OCT:10%PBS</td>
<td>Frozen for cryo-sectioning</td>
</tr>
<tr>
<td>Alternate Left Upper Lobe</td>
<td>Intact</td>
<td>Air Inflated, Vascular Contrast</td>
<td>To be determined</td>
<td>To be determined</td>
</tr>
<tr>
<td>Left Lower Lobe</td>
<td>Intact</td>
<td>PFA Fixed, Sucrose Cryopreserved, OCT embedded</td>
<td>Inflation fixed with 4% paraformaldehyde x 24 hr (Intact Lobe); Sectioned by grid into 0.5-1 cm3 pieces; Cryopreserved in 10% sucrose; Block in OCT</td>
<td>Inflation fixed, cryo-preserved and frozen for cryo-sectioning</td>
</tr>
<tr>
<td>Additional Tissue</td>
<td>Intact</td>
<td>Similar to Lung, see specific SOPs</td>
<td>These include thymus, spleen, esophagus, heart, nerve, etc; Block as appropriate for tissue</td>
<td>Formalin Fixed, Paraffin embedded, PFA/OCT embedded-frozen Single cell dissociation</td>
</tr>
<tr>
<td>Peripheral Blood</td>
<td>Whole</td>
<td>Cryopreserved, see specific SOPs</td>
<td>PBMC isolation, Plasma and Serum Storage</td>
<td>Cryopreserved</td>
</tr>
</tbody>
</table>

Table 1: Examples of LungMAP Human Tissue Core BioSpecimen Collection, Processing and Handling at Collection Site
Separation into lobes at bronchi

Each Lobe has a characteristic shape, that can be diagramed like this:

Left Lower Lobe Tissue Blocking Strategy
Right Lower Lobe Tissue Blocking Strategy

Lay slices down anterior/inferior to right

Inferior

Left Upper Lobe Tissue Blocking Strategy

Lay slices down anterior/inferior to right

Note, this example is not done correctly, all slices should be laid down in same direction

This example lobe was inflated with 50:50 OCT:10% PBS and then frozen over ethanol bath.

With Large Lobes, freeze strips of even numbered slices, block odd numbered slices
Right Middle Lobe Biopsy and Dissociation Strategy

Hilum with bronchi

Majority of RML dissociated to single cells

Biopsy from periphery

Right Middle Lobe Tissue Blocking Strategy - alternating slices flash frozen / FFPE / PFAFrozenOCT

Alternative
Slice fresh without inflation

Hilum with bronchi

Right Middle Lobe Tissue Blocking Strategy - alternating slices flash frozen / FFPE / PFAFrozenOCT
Trachea and Bronchi Tissue Blocking Strategy - image shows one side of bisected trachea only

Spleen Tissue Blocking Strategy

Turn/flip portion provided to approximate this orientation

Three large sections
A: Dissolution
B: Formalin
C: PFA

Lay slices down closest to you to the right
Then cut strips A, B...
Then, if needed, cut blocks 1, 2...