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603.3 & 604.5_URMC_HTC_Lung and Lobe Processing for SenNet

Forked from 603.3 & 604.5_URMC_HTC_Whole Lung and Lobe Processing

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Gloria S Pryhuber[1], Heidie Huyck[1]

[1]University of Rochester Medical Center

URMC Pryhuber Lab | Cellular Senescence Net...

Gloria S Pryhuber
University of Rochester Medical Center

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Protocol status: Working
We use this protocol and it's working

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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

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

MATERIALS

- 10X PBS; Diluted to 1X with DEPC H2O **Catalog #**Corning 46-013-CM
- 20% Paraformaldehyde (PFA); Diluted to 4% (m/v) PFA in 1X PBS **Catalog #**EMS 15713-S
- 10% Buffered Formalin **Catalog #**VWR 89370-094
- Diethylpyrocarbonate (DEPC) **Catalog #**Sigma D5758-100ML
- Ethanol **Catalog #**Koptec V-1001
- OCT (inflation); 50% (v/v) OCT in 1X PBS **Catalog #**Tissue-Tek 4583
- OCT (mold) **Catalog #**Tissue-Tek 4583
- Sucrose; 30% (m/v) Sucrose in 1X PBS **Catalog #**Sigma S9378-5KG
- Carboxymethylcellulose (CMC); 5% (w/w) CMC in DEPC H2O **Catalog #**Sigma C5678-1KG

Worksheet 603.A.2 HTC_Whole_or_Partial_Lung_Processing Worksheet

a.Grossing Station/ Biosafety Cabinet or Fume hood
b.Gloves, face protection and clothing consistent with blood and body fluid precautions
c.Heavy Duty Scissors and Wire Cutters
d.Biohazards Disposal Bag
e.Red Sharps Container
f.Biosafe surface for manipulation of lung
g.Small and Medium Gauze Pads – multi-pack
h.Standard Balance and Weigh Boats: Medium and Large
i.Airway Cannulas – Selection of sizes
  i.16 and 18 gauge angiocatheters (needles removed and discarded)
  ii.tracheal cannulas 2.0-4.0 OD
  iii.endotracheal tubes 2.5 – 8 mm OD; tubes >/= 4.0 cuffed
j.IV extension set tubing with clamp
k.20 ml syringe barrels for fixative
l.Needle free suture material
m.Zip Ties
n.Dissection Instruments: Same or similar to Sakura Series:
  1.#4791 Scalpel Handle with
  2.#4792 or #4793 Scalpel Blades, #61 (curved tip) or #62 (pointed tip), resp
  3.#4785 Trimming Blades, Short and/or #4789 Trimming Blades, Long
  4.#4786 Trimming Handle, Short and/or #4790 Long Straight
  5.#4794 Blade Scissors with #4796 Blades Sharp/Blunt
  6.#4807 Accu-Edge® Grossing Fork 2.5 mm
  7.#4800 Accu-Edge® Grossing Board *Inches (L x W x D):* 17 × 11.5 × 1
  8.#4802 Accu-Edge® Grossing Wells *Inches (L x W x D):* 12.9 × 3.25 × 2.15
  9.#8031 Slide Printer

a.#8033 Slide Unload Station
b.#8035 Ink Cartridge
c.#8040 Slide Magazine

10.Mopec Product #AB079 ProCUT Forcep Kit
o.Camera – iPad in water proof case works well
p.10% neutral buffered formalin – at least one liter with more on hand if needed for larger lungs
q.1xPhosphate Buffered Sodium (PBS diluted in DEPC treated water)
r.4% paraformaldehyde (prepared from 1:4 dilution of 16% stock non-buffered, no menthol formalin in 1x PBS in DEPC water)
s.Liquid Nitrogen in Dewar flask
t.Ring stand(s) and clamps (2 or more) – Fill syringe to 20 ml mark with fixative
u.Rulers – metal 18 inch (45cm) and 12 inch (30.5cm)

Troubleshooting

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

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?

Grossing of Tissues

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

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

11 Determine displacement volume without inflation

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

13 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 4 °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 4 °C to fix for 20-28 hours before moving to 30%Sucrose
d. Record these tissues and how processed in BRINDL database

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

15 Divide airways and vessels to obtain 5 independent lung lobes

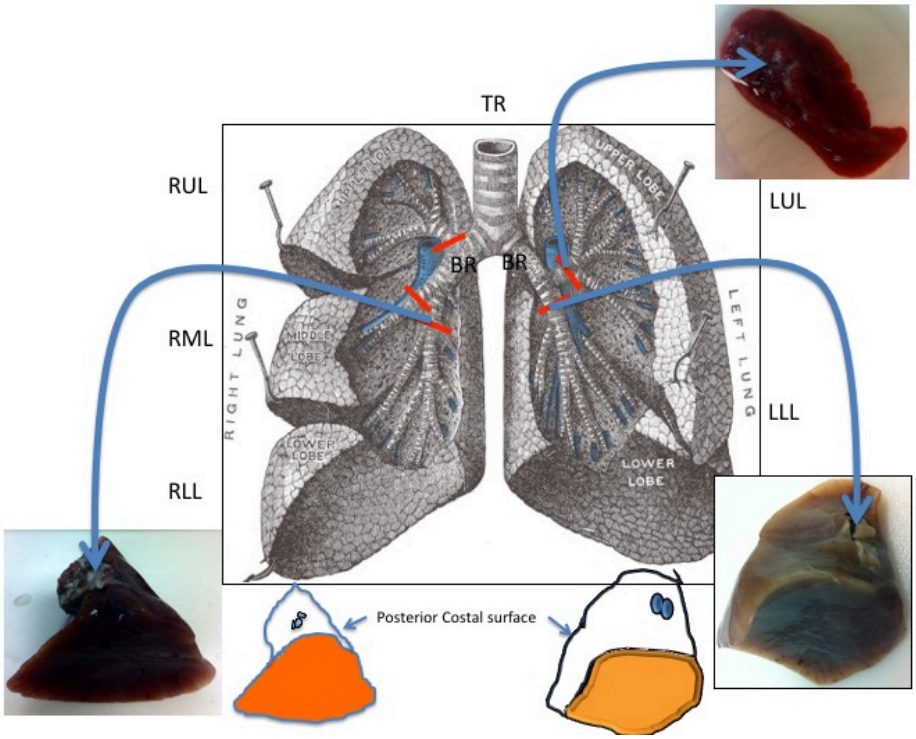
15.1 Weigh each lobe once isolated

15.2 Proceed with processing for each lobe as using appropriate Protocols

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

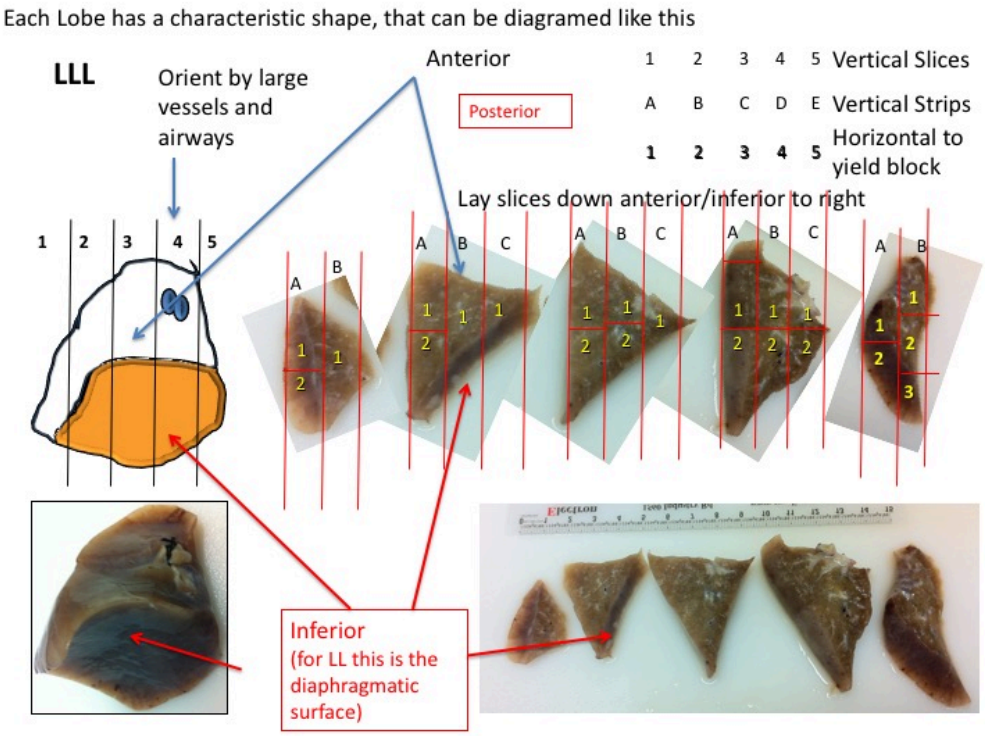
Lobe Sectioning Diagrams

17



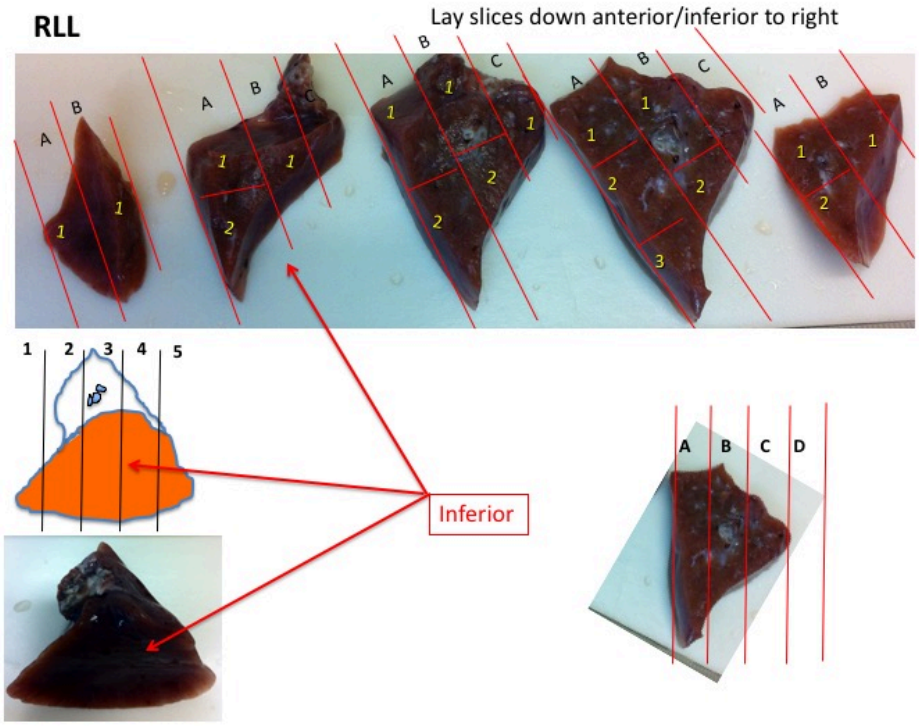
Separation into lobes at bronchi

18



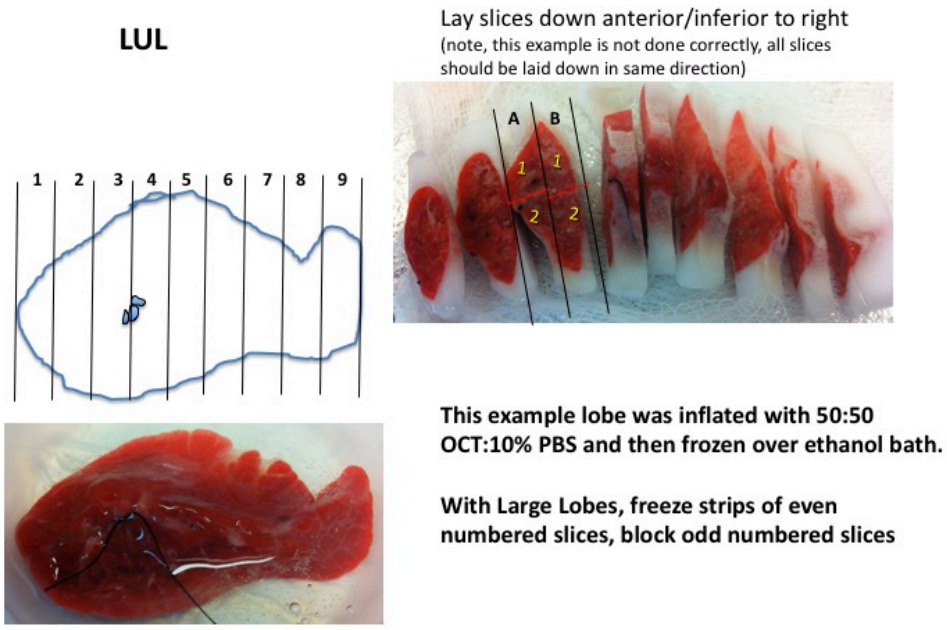
Left Lower Lobe Tissue Blocking Strategy

19



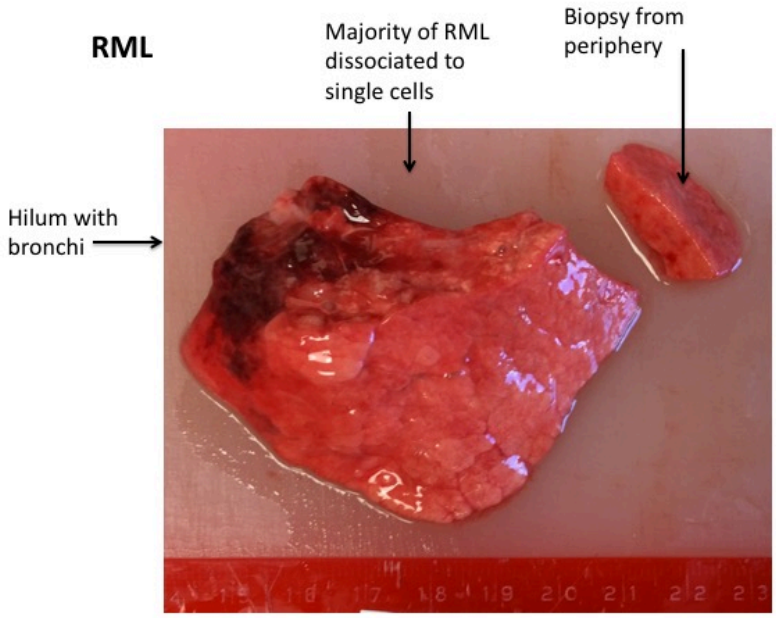
Right Lower Lobe Tissue Blocking Strategy

20



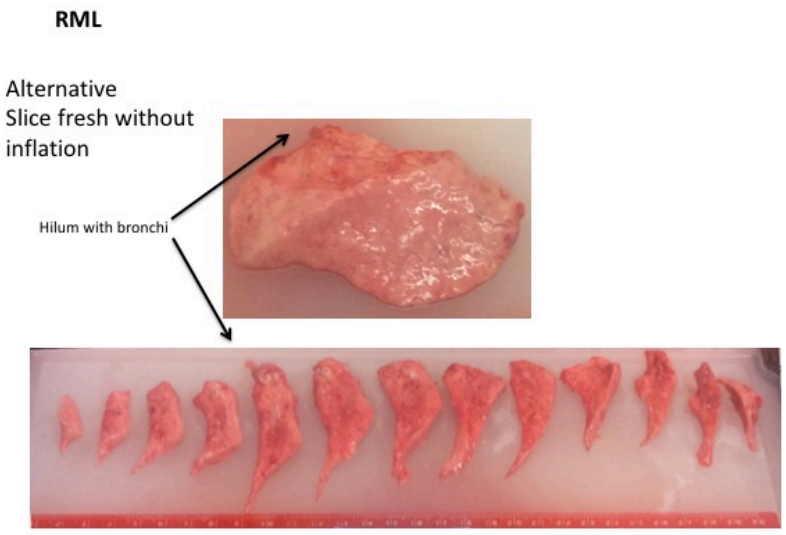
Left Upper Lobe Tissue Blocking Strategy

21



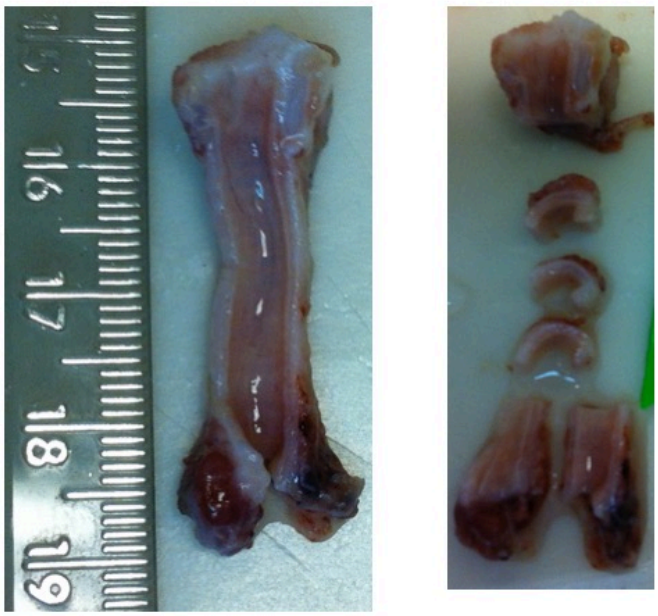
Right Middle Lobe Biopsy and Dissociation Strategy

22



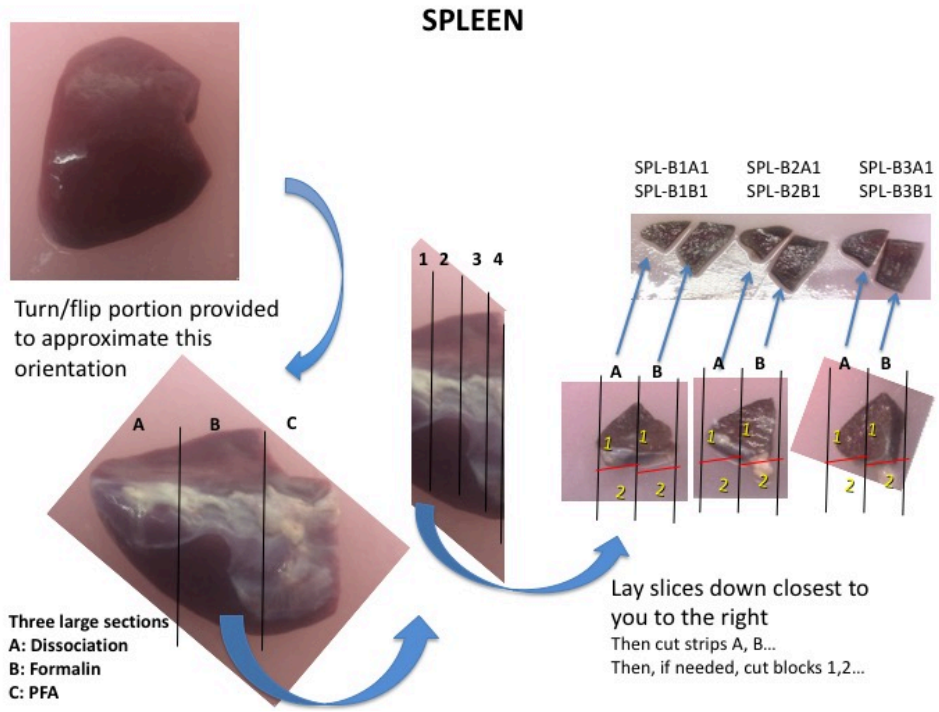
Right Middle Lobe Tissue Blocking Strategy - alternating slices flash frozen / FFPE / PFAFrozenOCT

23



Trachea and Bronchi Tissue Blocking Strategy - image shows one side of bisected trachea only

24



Spleen Tissue Blocking Strategy