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603.3 & 604.5_URMC_HTC_Whole Lung and Lobe Processing V.2

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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)

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.

Ethics statement

The protocols.io team notes that research involving animals and humans must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Ethics Committee or Board.

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?

CT Scan

9 CT Imaging

Donor lungs arrived with the trachea occluded by tie or staple. The trachea was opened and intubated with an endotracheal tube that was then held in place with a plastic zip tie. In some cases, the trachea was too short for intubation or the intact lungs too large for the field of view. In this case the main bronchi were separated and each lung was inflated and scanned individually. The lungs were kept cold and moist in a plastic bag on ice prior to scanning. Lungs were inflated with air (21% oxygen) as uniformly as possible, massaging gently as needed to reverse atelectasis, to a pressure of 25 cm H20 using a Neopuff T-piece Resuscitator (Fisher & Paykel Healthcare, Auckland, New Zealand). All CT scans were performed at this steady distending pressure on a clinical CT scanner (Brilliance 64, Philips North America Corporation, Cambridge, MA), with the lungs lying supine on a bed of cotton or air pillows in as close to anatomical position as could be maintained. A helical scan protocol was used with 120 kVp, 198 mAs, slice thickness of 0.67 mm, and a spiral pitch factor of 0.348. The YF (ultra-high resolution) kernel was used to reconstruct 1024×1024 images over the whole CT stack. Depending on the physical size of a particular lung, this resulted in in-plane resolution of 0.09-0.24 mm. Images were uploaded from the scanner to the University of Rochester Medical Center's Picture Archiving and Communication System (PACS) in the DICOM format for secure storage and retrieval.

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 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

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.

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	Organ	Starting Tissue	Prep	Process	BioSpecimen Type Collected from Organ
	Trachea and large bronchi	Intact	Paraffin and OCT Frozen	Bisect longitudinally; Section into <1 cm long half rings	For mali n

					Fixed, Paraffin embedded PFA SOCT embedded and frozen
	Alternate Trachea and large bronchi	Intact	Cell Isolates	Enzymatic digestion and cell isolation	Frozen isolated cell aliquots
	Lung: Right Upper Lobe +/- Right Middle Lobe	Fresh Tissue Blocks	Mixed Dissociated Cells	Dissociate cells by mechanical and enzymatic digestion procedure	Cryopreserve mixed cell aliquots
	Sorted Dissociated Cells	Flow Activated Cell Sorting dissociated cells	Cryopreserve sorted cell aliquots “ex. Endothelial, Epithelial, Fibroblast, Bone Marrow Derived (CD45+) Separation”		
	Cryo-preserved QI	Cryo-preserved cells	Recover frozen cell aliquots, test purity by PCR, culture for viability and proliferative/ differentiation potential		
	Add’l Right Middle Lobe	Intact - Biopsies	Flash Frozen	Stored at -80	Protein Homogenates, RNA

					and/or DNA Isolation
	Alternate Right Middle Lobe	Intact	Formalin Inflation, Paraffin	Inflation fixed with 10% buffered formalin x 24 hr (Intact Lobe); Sectioned by grid into 0.5-1 cm3 pieces; Dehydrated to 70% Ethanol	Inflation fixed, paraffin embedded ready for sectioning
	Alternate Right Middle Lobe	Fresh Tissue Blocks	Fresh Frozen Embedded in OCT/CMC/none	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	Non-inflated, non-fixed flash frozen tissue blocks, mapped to originating location
	Right Lower Lobe	Intact	Formalin Inflation, Paraffin	Inflation fix with 10% buffered formalin x 24 hr (Intact Lobe) Sectioned by grid into 0.5-1 cm3 pieces Dehydrated to 70% Ethanol	Inflation fixed, paraffin embedded ready for secti

					onin g
	Left Upper Lobe	Intact	PFA Fixed, Sucrose Cryopreserved, OCT embedded	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	Infla tion fixed , cryo - pres erve d and froz en for cryo - secti onin g
	Alternate Left Upper Lobe	Intact	OCT/PBS	Inflate with 50:50 OCT:10%PBS	Froz en for cryo - secti onin g
	Alternate Left Upper Lobe	Intact	Air Inflated, Vascular Contrast	To be determined	To be dete rmin ed
	Left Lower Lobe	Intact	PFA Fixed, Sucrose Cryopreserved, OCT embedded	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	Infla tion fixed , cryo - pres erve d and froz en for cryo

					- sectioning
	Additional Tissue	Intact	Similar to Lung, see specific SOPs	These include thymus, spleen, esophagus, heart, nerve, etc; Block as appropriate for tissue	Formalin Fixed, Paraffin embedded, PFA/OCT embedded-frozen Single cell dissociation
	Peripheral Blood	Whole	Cryopreserved, see specific SOPs	PBMC isolation, Plasma and Serum Storage	Cryopreserved

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

Lobe Sectioning Diagrams

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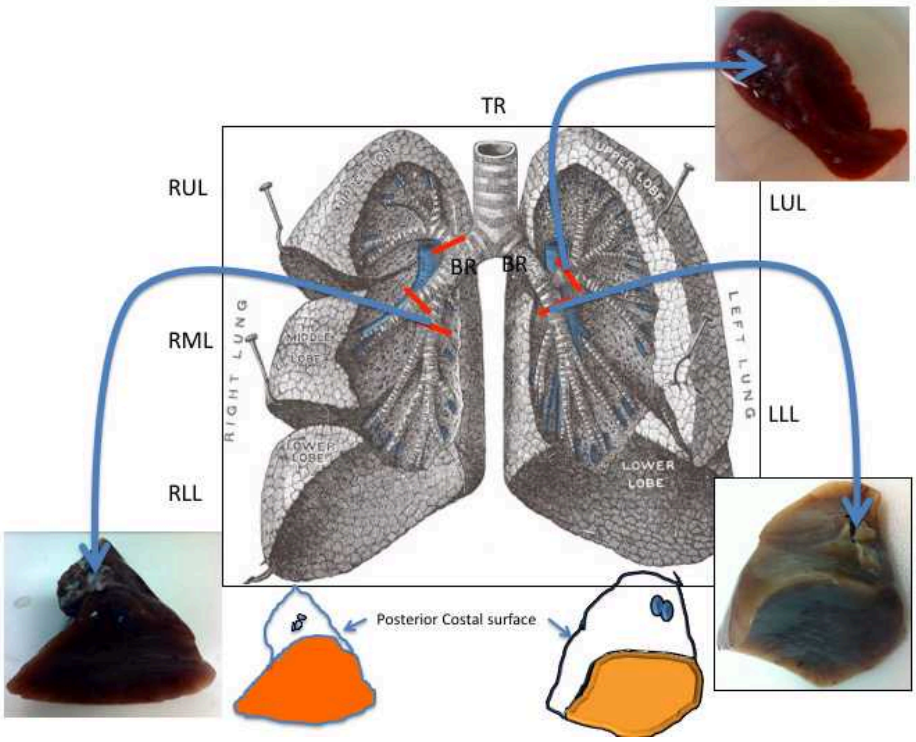
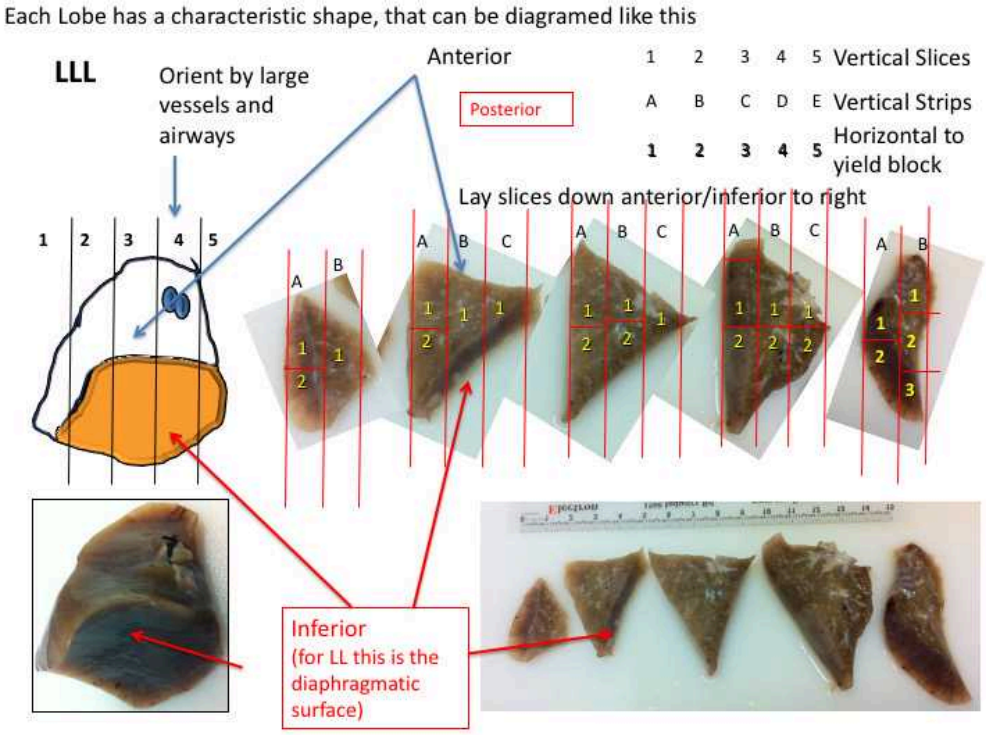


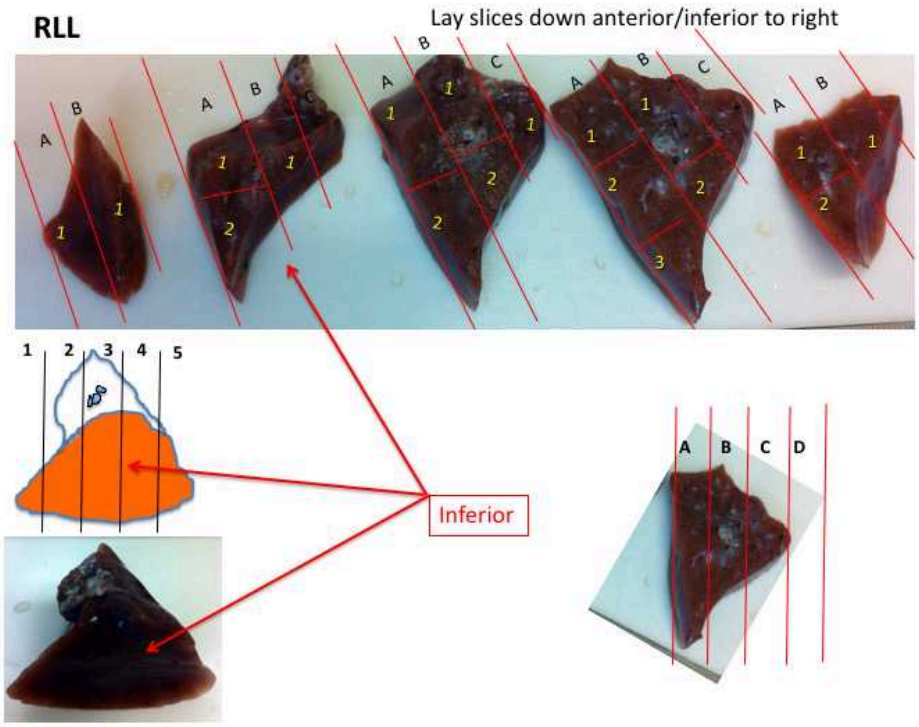
Separation into lobes at bronchi

20



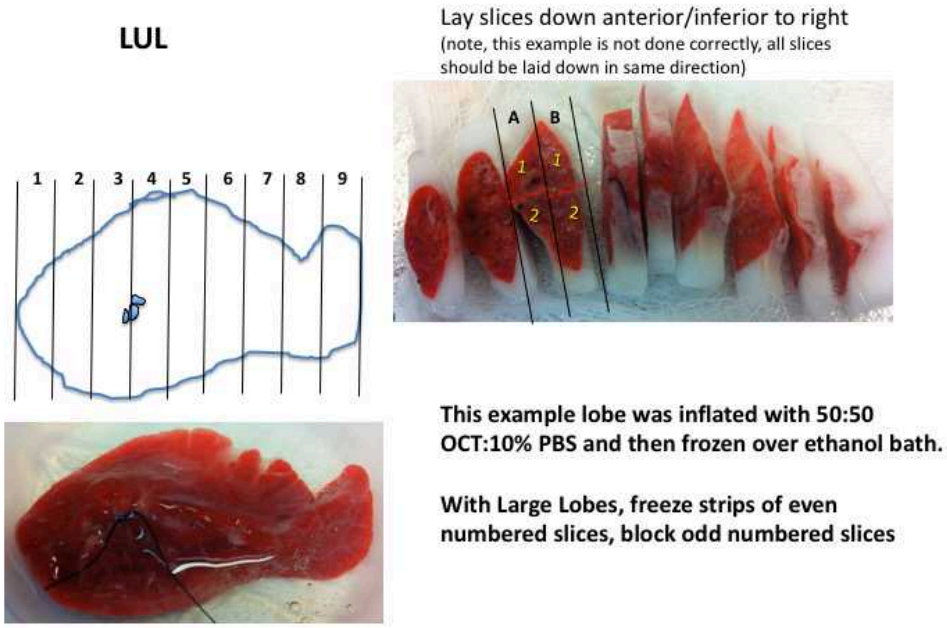
Left Lower Lobe Tissue Blocking Strategy

21



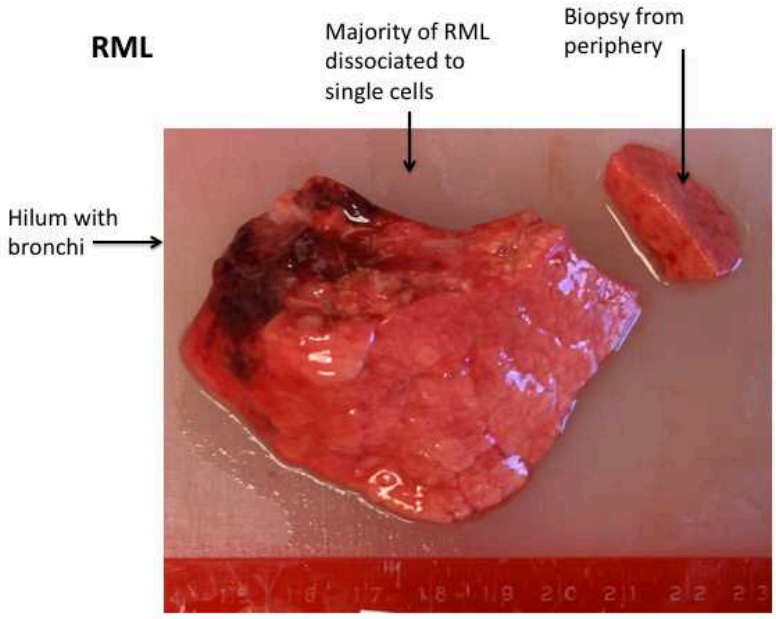
Right Lower Lobe Tissue Blocking Strategy

22



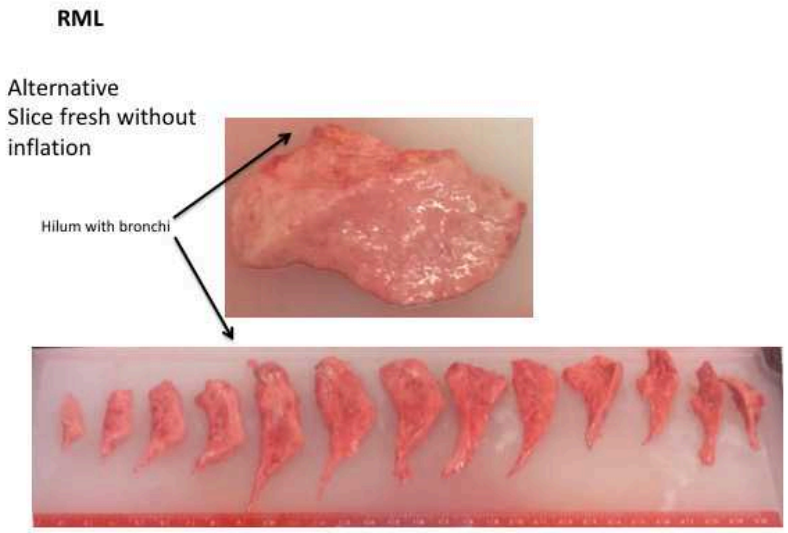
Left Upper Lobe Tissue Blocking Strategy

23



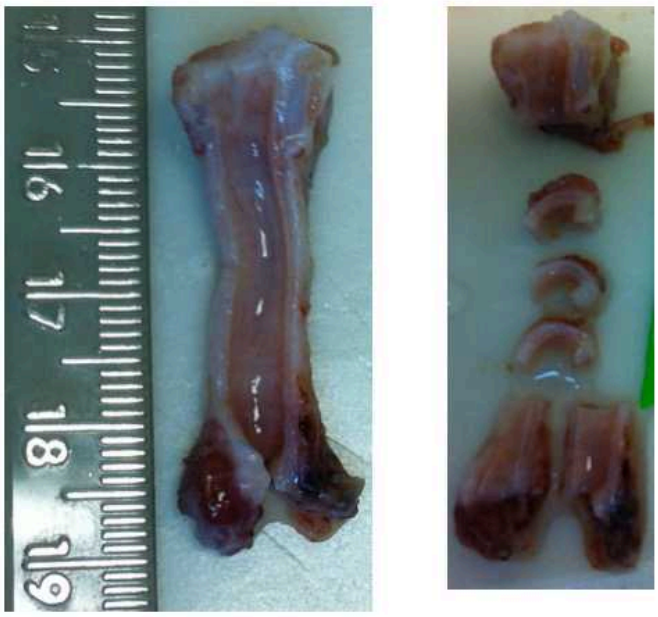
Right Middle Lobe Biopsy and Dissociation Strategy

24



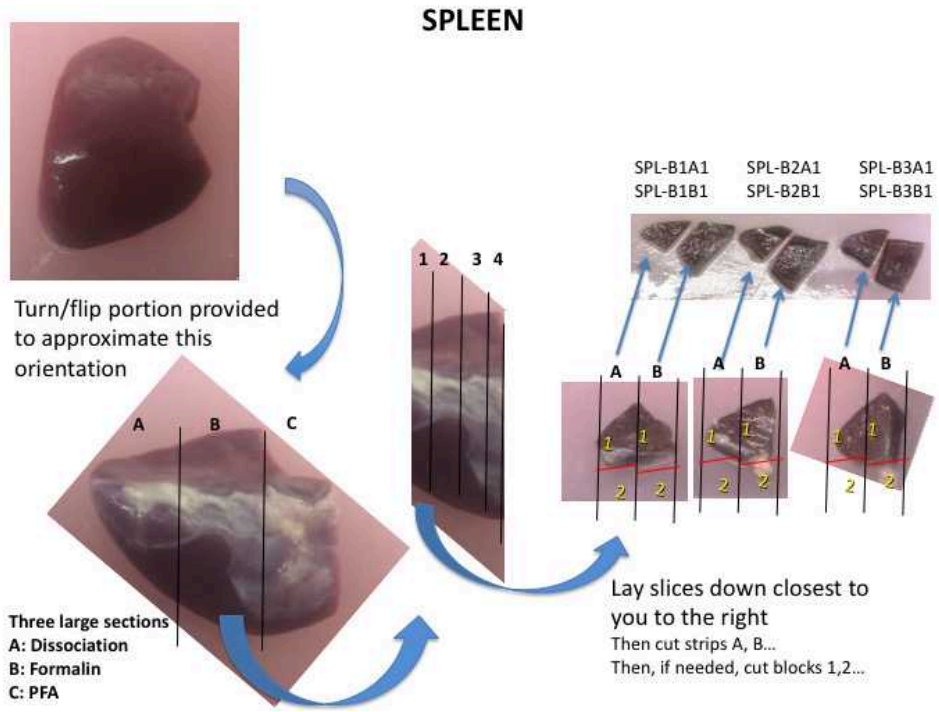
Right Middle Lobe Tissue Blocking Strategy - alternating slices flash frozen / FFPE / PFAFrozenOCT

25



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

26



Spleen Tissue Blocking Strategy