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# 611.2 URMHC HTC Formalin-Inflated, Paraffin-Embedded Human Lung Tissue

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Human BioMolecular Atlas Program (HuBMAP) Method Development Community

LungMap2 Consortium 1



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Processing of Formalin Fixed Lung and Non-Lung Tissue for the LungMAP HTC

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Inflation fixation, lung, FFPE



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## Worksheet 603.A.2 HTC\_Whole\_or\_Partial\_Lung\_Processing

Biosafe surface and environment for manipulation of lung  
Grossing Station, Biosafety Cabinet or Fume Hood

1. Gloves, face protection and clothing consistent with blood and body fluid precautions
2. Biohazards Disposal Bag
3. Red Sharps Container
4. Tissue mega-cassettes (optional) and uni-cassettes
5. Scalpels and Trimming Blades with Handles; Forceps; Other Dissection Equip
6. Labeling pencil
7. 10% neutral buffered formalin – at least one liter, with more on hand if needed for larger lungs
8. 1xPhosphate Buffered Sodium
9. 30% Ethanol; 50% Ethanol and 70% Ethanol. All solutions are made with Diethylpyrocarbonate (DEPC) treated water or directly treated with DEPC to minimize RNA degradation
10. Small and Medium Gauze Pads – multi-pack
11. Standard Balance and Weigh Boats: Medium and Large
12. 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  $\geq$  4.0 cuffed
14. IV extension set tubing with clamp
15. 20 ml syringe barrels for fixative
16. Needle free suture material

Working with human tissues: 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 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.

### Record Process

- 1 Record details of procedure in Worksheet or Directly in Inventory or ELN
- 2 Keep photographic recording of inflation and blocking of lung lobe and tissue

## Inflation Fixed (Formalin) Lung Lobe

- 3 Inflation fixation procedure should be accomplished in a grossing station or biological safety cabinet with the operator taking appropriate blood and body fluid precautions.
- 4 Keep the surface of the lobe / tissue moist at all times
- 5 Connect a short piece of IV extension tubing, clamped with hemostat or shut off with stopcock, to a 10 or 20 ml syringe barrel (without plunger) suspended in a clamp on a ring stand so that the 5 ml syringe mark is 25 cm above the level of the main airway in the lobe to be fixed.
- 6 Fill the syringe barrel with 10% Neutral Buffered Formalin (NBF)
- 7 Flush the IV extension tubing through with formalin to remove air bubbles and so the NBF fluid level is at 10 or 20 ml mark on the syringe. Reclamp. prior to connection to the instillation cannulas
- 8 Cannulate the main bronchus, identified by its cartilage containing wall, with an endotracheal tube or 16 or 18 gauge catheter.  
  
Fix the airway tube in place with suture material or zip tie.  
  
If two bronchi are apparent due to a branch-point, each can be cannulated.  
  
Tying in of the airway tubes is the “trickiest” part of the entire procedure and is most easily done by two people.
- 9 Connect the IV extension tubing coming from the syringe to the airway tube(s).  
  
If more than one bronchi is cannulated, they can be instilled serially or simultaneously with a second syringe set up.
- 10 Release the clamp and allow formalin to infuse into the lung lobe that should visibly inflate, the degree of inflation varies.

Transient, gentle pressure on the fluid head may be needed to start the flow of fixative.

Add NBF to the syringe to maintain meniscus at 25 cm water inflation pressure. Keep track of

- 11 volume added.  
  
Maintain instillation pressure for approximately 10 minutes after fluid stops flowing.
- 12 Document volume of fixative infused and comment on any leakage (with degree -minimal, moderate, severe)
- 13 Photograph anterior and posterior sides before and after inflation.
- 14 Once instillation is complete, pull the suture or zip tie that is on the airway and airway cannula tight while removing the cannula.  
  
This is best done with a second pair of hands. Also helpful to have a hemostat available as an emergency clamp.
- 15 Place the securely tied lung lobe in a specimen container filled with 10% NBF.  
  
For good fixation, prefer with a volume ratio of 10:1 fixative:lung.
- 16 Place in the cold room for 20-24 hours
- 17 Twenty to 24 hours after fixation begins, divide the lobe as diagramed in attached figures into approximately **0.5 cm** thick, 1.0 x 1.0 cm<sup>2</sup> cubes, placing each cube in an appropriately labeled tissue cassette
- 18 Place back in 10% formalin in the cold for 18-24 hours.
- 19 The tissue cubes are then rinsed in 1xPBS and dehydrated by 20-minute successive dwell times in PBS, 30% EtOH, 50% EtOH and 70% EtOH made with diethylpyrocarbonate (DEPC) treated water.
- 20 Tissues may be stored in 70% EtOH in cold until further processed into paraffin.

## Formalin Fixed Non-Lung Tissue

- 21 Remove excess tissues around trachea and large airways at hilum
- 22 Collect lymph nodes, excess large vessels, esophagus, spleen and nerves (Best to place these back in shipping buffer and Tend to Lung First)
  - 22.1 Weigh each tissue collected
  - 22.2 The non-lung samples are sectioned into approximately 0.5-1 cm<sup>3</sup> portions
- 23 Divide these tissues between 10 % Formalin and PFAS-Cryoprotection

Record these tissues and how processed in BRINDL database

  - 23.1 For 10% formalin fixation, place in labeled tissue cassette in formalin; stored at 4<sup>0</sup>C to fix for 40-48 hours. Volume of fixative to tissue should be approximately 10:1.

Forty-48 hours after fixation begins, the tissues are rinsed in 1xPBS and dehydrated by 20 minute successive dwell times in PBS, 30% EtOH, 50% EtOH and 70% EtOH made with diethylpyrocarbonate (DEPC) treated water.

Tissues may be stored in 70% EtOH in cold until further processed in paraffin
  - 23.2 For Freezing in OCT: fix in 4% PFA x 20-24 hrs, cryo-protect in 30% sucrose and continue to process for freezing in OCT along with lung tissue to be frozen (See PFA fixed, sucrose cryoprotection protocol)

## Tissue Processor

- 24 From 70% ethanol, place cassettes into metal basket in VIP processor's retort (up to 150 unicassettes or 75 megacassettes) in 70% ethanol already in the retort

- 25 Use overnight program #3 on VIP Processor
- 70% Ethanol – 1 hour – ⌚ 40 °C
  - 80% Ethanol – 1 hour – ⌚ 40 °C
  - 95% Ethanol – 1 hour – ⌚ 40 °C
  - 95% Ethanol – 1 hour – ⌚ 40 °C
  - 100% Ethanol – 1 hour – ⌚ 40 °C
  - 100% Ethanol – 1.5 hours – ⌚ 40 °C
  - Histological grade Xylene – 1 hour – ⌚ 40 °C
  - Histological grade Xylene – 1.5 hours – ⌚ 40 °C
  - McCormack's Paraplast (paraffin) – 1 hour – ⌚ 60 °C
  - McCormack's Paraplast (paraffin) – 1 hour – ⌚ 60 °C
  - McCormack's Paraplast (paraffin) – 1.5 hours – ⌚ 60 °C

- 26 Remove cassettes out of processor to embed

#### Embedding Tissue

- 27 Place cassettes in holding tank in the embedding center until paraffin is melted (~15 minutes)
- 28 Embed tissue in metal mold as it was in the processing cassette
- 29 Cool and solidify blocks on cold plate on embedding center

- 30 When blocks “pop” remove them from molds.
- 31 Clean outside of cassettes of excess wax and trim excess wax from blocks with the paratrimmer
- 32 Store blocks at room temperature away from heat and sunlight

#### Sectioning Paraffin Embedded Tissue

- 33 Cool blocks on ice
- 34 Using microtome, trim into block at a higher thickness (~14 microns) until a full section is achieved.
- 35 Cool block on ice
- 36 Section blocks at 4-5 microns into a ribbon
- 37 Place ribbon into cool water bath
- 38 Place section on slide

- 39 Place section into hot water bath
- 40 Melt section onto slide
- 41 Dry slide standing up in rack overnight or until dry
- 42 Store slides in slide boxes at room temperature away from heat and sunlight