616.1 URMC HTC BSL2+ Formalin-Inflated, Paraffin-Embedded Human Lung Tissue V.3

Version 1 is forked from 611.2 URMC HTC Formalin-Inflated, Paraffin-Embedded Human Lung Tissue

Gloria S Pryhuber[1], Heidie Huyck[1], Lisa Rogers[2]

[1]University of Rochester Medical Center; [2]University of Rochester

Human BioMolecular Atlas Program (HuBMAP) Method Development Community

LungMap2 Consortium

1 more workspace

Gloria S Pryhuber
University of Rochester Medical Center

VERSION 3

MAR 28, 2024

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.kxygxejwdv8j/v3

Protocol Citation: Gloria S Pryhuber, Heidie Huyck, Lisa Rogers 2024. 616.1 URMC HTC BSL2+ Formalin-Inflated, Paraffin-Embedded Human Lung Tissue. **protocols.io** https://dx.doi.org/10.17504/protocols.io.kxygxejwdv8j/v3Version created by Gloria S Pryhuber

ABSTRACT

Processing of Formalin Fixed Lung and Non-Lung Tissue for the LungMAP HTC and includes acquisition of limited Control tissue from Surgical Pathology.

GUIDELINES

Scientific Principles or Validation of Procedures :

1. CDC. “COVID-19 Personal Protective Equipment (PPE) for Healthcare Personnel.” *Centers for Disease Control and Prevention*, 1 Jan. 2020, www.cdc.gov/coronavirus/2019-ncov/downloads/COVID-19-PPE.pdf.
2. WHO. “Personal Protective Equipment for COVID-19.” *World Health Organization*, World Health Organization, 5 May 2020, www.who.int/medical_devices/priority/COVID_19_PPE/en/.
3. University of Rochester Medical Center, director. *COVID-19 Safety Training*, University of Rochester Medical Center , 15 June 2020, rochester.csod.com/LMS/LoDetails/DetailsLo.aspx?loid=e8469bef-ae75-4d87-9ec1-f5d33e4f1519&query=%3fq%3dCOVID-19+Safety+Training&isCompletionRedirect=true&loStatus=16®num=1#t=1.

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

Created: Mar 28, 2024

Last Modified: Mar 28, 2024

PROTOCOL integer ID: 97497

Keywords: Inflation fixation, lung, FFPE, COVID-19, SARS CoV2

Funders Acknowledgement:
Lung Molecular Atlas Program
Grant ID: U01HL148861
Human Biomolecular Atlas Program
Grant ID: U54HL165443

MATERIALS

Worksheet 603.A.2 HTC_Whole_or_Partial_Lung_Processing

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

1. N95 masks, face shields, double gloves, shoe covers, hair nets, sleeve covers, and lab coat for PPE, all consistent with recommended PPE for COVID-19
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 >/= 4.0 cuffed
14. IV extension set tubing with clamp
15. 20 ml syringe barrels for fixative
16. Needle free suture material
17. Bleech
18. Oxivir Tb or simlar anti-viral

SAFETY WARNINGS

Working with potentially or known SARS CoV2/COVID-19+ human tissue: Personnel will adhere to safe work processes all consistent with recommended PPE for COIVD-19 work. PPE will be used including N95 mask, face shield, lab coat, closed shoes and double gloves, shoe covers, hair net, and sleeve covers. 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 all steps of sectioning and blocking of lung lobe and tissue

Inflation Fixed (Formalin) Lung Lobe

3 Keep the lobe / tissue cold and the surface moist at all times

4 Inflation fixation procedure should be accomplished in a biosafety cabinet with the operator taking appropriate fixative, blood and body fluid, including COVID-19, precautions.

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.

11 Add NBF to the syringe to maintain meniscus at 25 cm water inflation pressure, equilibrating at the 5ml syringe mark used to measure the height of the syringe barrel in step 5. Keep track of 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 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.

14 Photograph anterior and posterior sides before and after inflation.

15 Place the securely tied lung lobe in a specimen container filled with 10% NBF.

For good fixation, prefer a volume ratio of 10:1 fixative:lung.

16 Place in the cold room for 40-48 hours

Once tissue has been in fixative for 40-48 hours, processing may continue on grossing station or fume hood

17 Forty to 48 hours after fixation begins, divide the lobe as diagrammed in attached figures into approximately 0.5 cm thick, 1.0 x 1.0 cm^2 cubes, placing each cube/block 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, with agitation on a rotating platform at room temperature, in PBS, 30% EtOH, 50% EtOH and 70% EtOH made with diethylpyrocarbonate (DEPC) treated water.

20 Tissues should be stored in 70% EtOH at 4 deg C for a minimum of overnight, or longer, until further processed into paraffin.

Formalin Fixation of Lung Tissue without Instillation of Formalin

21 Place the lung slice that is to be formalin fixed in an approximately 10:1 volume of 10% buffered formalin in a leak proof container, a 200 ml specimen cup works well.

22 Shake the tightly closed container vigorously by hand for 1 minute to encourage self-inflation of the lung tissue.

23 Agitate the tissue in the container on a rotating platform for 60-120 minutes.

24 Store the tissue-containing container at 4deg C x 40-48 hrs and then cut the slice into approximately 1cm x 1cm x 0.5 cm blocks. Place the blocks in labelled tissue cassettes and place these back in the formalin for an additional 24 hours at 4 deg C

25 The tissue cubes are then rinsed in 1xPBS and dehydrated by 20-minute successive dwell times, with agitation on a rotating platform at room temperature, in PBS, 30% EtOH, 50% EtOH and 70% EtOH made with diethylpyrocarbonate (DEPC) treated water.

26 Tissues should be stored in 70% EtOH at 4 deg C for a minimum of overnight, or longer, until further processed into paraffin.

Formalin Fixed Non-Lung Tissue

27 Remove excess tissues around trachea and large airways at hilum

28 Collect lymph nodes, excess large vessels, esophagus, spleen and nerves (Best to place these back in shipping buffer and Tend to Lung First)

28.1 Weigh each tissue collected

28.2 The non-lung samples are sectioned into approximately 0.5-1 cm3 portions

29 Divide these tissues between 10 % Formalin and PFAS-Cryoprotection

Record these tissues and how processed in BRINDL database

29.1 For 10% formalin fixation, place in labeled tissue cassette in formalin; stored at 4^0C to fix for 64-72 hours (consistent with lung tissue fixation time). Volume of fixative to tissue should be approximately 10:1.

The tissue cubes are then rinsed in 1xPBS and dehydrated by 20-minute successive dwell times, with agitation on a rotating platform at room temperature, in PBS, 30% EtOH, 50% EtOH and 70% EtOH made with diethylpyrocarbonate (DEPC) treated water.

Tissues should be stored in 70% EtOH at 4 deg C for a minimum of overnight, or longer, until further processed into paraffin.

29.2 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

30 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

31 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

32 Remove cassettes out of processor to embed

Embedding Tissue

33 Place cassettes in holding tank in the embedding center until paraffin is melted (~15 minutes)

34 Embed tissue in metal mold as it was in the processing cassette

35 Cool and solidify blocks on cold plate on embedding center

36 When blocks “pop” remove them from molds.

37 Clean outside of cassettes of excess wax and trim excess wax from blocks with the paratrimmer

38 Store blocks at room temperature away from heat and sunlight

Sectioning Paraffin Embedded Tissue

39 Cool blocks on ice

40 Using microtome, trim into block at a higher thickness (~14 microns) until a full section is achieved.

41 Cool block on ice

42 Section blocks at 4-5 microns into a ribbon

43 Place ribbon into cool water bath

44 Place section on slide

45 Place section into hot water bath

46 Melt section onto slide

47 Dry slide standing up in rack overnight or until dry

48 Store slides in slide boxes at room temperature away from heat and sunlight

Control FFPE tissue samples from other source

49 Occasionally, completely de-identified tissue samples are obtained from the surgical pathology lab to be used as assay control or comparison tissue samples. These could include tonsil, thymus, heart, lung and other thoracic or abdominal tissues.

50 These control tissues are received as FFPE blocks prepared by standard protocols of the surgical pathology lab with estimates of dates and times processed from patient retrieval through formalin, ethanol and embedding in paraffin and reported as metadata with datasets. They are used for non-identifiable photomicroscopy and imaging. Nucleotide sequence is not generated.