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HuBMAP lung tissue sampling protocol

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We use this protocol and it's working

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Abstract

Purpose and Scope of the Procedure:
-Safe work with unfixed lung tissue potentially carrying infectable organisms.
-Rapid blocking, embedding, and freezing of human lung tissue in no freezing media or 100% OCT.

Attachments

PDF HuBMAP lung tissue s... 116KB

PDF HuBMAP lung tissue s... 1.1MB

Guidelines

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. 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%3dCOVID19+Safety+Training&isCompletionRedirect=true&loStatus=16®num=1#t=1.

Materials

Materials:

- Brady FREEZERBONDZ Polyester thermal transfer printer labels Fisher Scientific Catalog #22-500521
- OCT (mold) Contributed by users, Catalog #Tissue-Tek 4583
- Worksheet 603.A.HTC_Whole_or_Partial_Lung_Processing
- Biosafe surface and environment for manipulation of lung
- Biosafety Cabinet or Grossing Station

1. N95 masks, face shields, double gloves, shoe covers, hair nets, sleeve covers, and labcoat for PPE, all consistent with recommended PPE for SARS-CoV2 / COVID-19 work
2. Biohazards Disposal Bag
3. Red Sharps Container
4. Small and Medium Gauze Pads – multi-pack
5. Plastic Cryomolds
6. Scalpels and Trimming Blades with Handles; Forceps; Other Dissection Equipment
7. Freezerbondz Labels and Printer
8. Standard Balance and Weigh Boats: Medium and Large
9. Rulers – metal 18 inch (45cm) and 12 inch (30.5cm)
10. 100% OCT (Tissue Tek)
11. Flat ice buckets or large Styrofoam boxes partially filled with dry ice pellets
13. Flat metal pans
14. Aluminum foil and labeling tape or freezer storage bags
15. Cryovials with red cap inserts
16. Small biohazard stickers

Safety warnings

Working with human tissue potentially carrying infectious organisms: All laboratories should perform a site-specific and activity-specific risk assessment and follow standard precautions when handling clinical specimens. Personnel will adhere to safe work standard protocol, including N95 mask, face shield, lab coat, closed shoes and double gloves, shoe covers, hair net, and sleeve covers. All activity will be behind the 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 institutional biosafety committee approved protocol. All institutional biosafety measures are followed in any manipulation of these human tissues.

Unpacking

1 Open shipped package on arrival to remove the blood sample, if provided, as it should be kept at room temperature.

2 Store the remainder of the package in a cold room to maintain the tissue at approximately 4 °C until processing begins.

3 Assign BRINDL PID to tissues and images by clicking the “arrived” button in the BRINDL Screening log.

4 Record Information from shipping labels in the database or worksheet for entry ASAP.
· UNOS #; Referring Company #
· Courier and Tracking Numbers

5 Remove shipping labels from the package and set them aside for the sample file.

6 Open shipping box and remove styrofoam cover. Record the condition of packing in the database (e.g., ice sufficient, layered packaging, no leakage, etc.)

7 Open the container containing the tissue and transfer the lung tissue to a grossing station or in a fume hood.
a. Record condition of lung tissue in the database
b. Is the tissue submerged (usually in a plastic bag inside a hard plastic container)?
c. Are trachea and both lungs included and intact?
d. Is trachea occluded? If so, how?
e. Are the thymus, spleen, blood sample, lymph nodes included?

Fresh Tissue Frozen in Cryomolds and No Freezing Media (flash frozen)

8 Conduct slicing and blocking procedure at a grossing station or in a fume hood, ensuring appropriate blood and body fluid precautions are followed.

9 Prepare dry ice in an ice bucket or styrofoam box. Add more dry ice as needed to maintain rapid freezing of the molds.

10 Keep the surface of the lung lobes moist and cool at all times.

11 Place fresh left upper and lower lobes on the cutting surface and section the tissue into slices and blocks (Fig. 1A). Our standard is approximately 1cmx 1cm x 0.5 cm thickness of tissue, including airway and accompanying blood vessels, for 4 serial slices for different types of fixation or flash freezing from 9 distinct lung regions as outlined in Table 1.
- 2 slices for fresh flash freezing
- 1 slice for fresh frozen embedded in OCT
- 1 slice for formalin fixation followed by paraffin embedding.

12 To dissect distinct left upper lung regions, we begin with the extrapulmonary bronchus (Region 1, Fig. 1B). Next, we carefully isolate the 3^{rd} or 4^{th} generations bronchus from the surrounding lung parenchyma and dissect the airway tissues (Region 2, Fig. 1C). We then make a 5 cm incision from the pleura to locate a bronchiole (~ 2 mm in diameter) and dissect it along with the accompanying pulmonary structures (Region 3, Fig. 1D). Similarly, we make a 2 cm incision from the pleura to identify bronchioles (< 1 mm in diameter) and dissect them (Region 4, Fig. 1E). Finally, we trim the lung edge to isolate alveolar tissue with pleura (Region 5, Fig. 1F). Dissection of Regions 6~9 follows the same approach as used for the left upper lung lobe.

13 Duplicate samples will be collected from anatomically similar regions of the lung. Each sample is appropriately labeled as outlined in Table 2. Work quickly to avoid warming the lung tissue. Photograph tissue sections to document their location, block layout, and processing method applied.

14 For OCT embedding: Fill the cryomolds with a thin layer of embedding medium. Position the tissue in the labeled cryomolds, ensuring there is a small amount of embedding medium surrounding the tissue.

15 Gently press down on the tissue with forceps to remove as much air as possible and ensure the tissue lies on the bottom of the cryomold during freezing, which will facilitate sectioning. Place cryomolds containing tissue on the metal pan on dry-ice and allow them to freeze while adding more freezing medium (OCT) to completely cover the embedded tissue.

16 While cryomolds are freezing, print out Freezerbondz labels for each block and place the label on middle of a piece of aluminum foil along with a small biohazard sticker. Apply the label to the foil prior to wrapping the cold blocks to improve adhesion and minimize warming during the wrapping process.

17 Once frozen, quickly wrap each block with the correctly labeled aluminum foil and return to the frozen metal pan to keep frozen until transferred to -80°C freezer.

18 Collect wrapped cryomolds into labeled freezer bags, place them in cold labeled freezer boxes, and store them in -80°C freezer.

19 For fresh flash frozen samples, section the tissue into 0.25-1.0 cm^3 portions and place them into properly Freezerbondz labeled freezer vials.

20 The weight of the collected tissue is recorded.

21 Freeze freezer tubes containing individual samples in liquid nitrogen and transfer them to -80C freezer.

Formalin-fixed tissue followed by paraffin-embedding and H&E staininged section

22 Section the tissue into 1cmx 1cm x 0.5 cm thick pieces.

23 Transfer the tissue to a container filled with 10% neutral buffered formalin.

24 Gently agitate the container on a shaker at room temperature for 72 hours to ensure proper fixation. If the formalin turns yellow due to tissue effusion after 24 hours, replace it with fresh formalin.

25 After 72 hours, wash the tissues in tap water for 10 mins, repeating this process three times.

26 Store the tissues in 70% ethanol after washing and send them to the histology core.

27 Formalin-fixed tissues are processed in a Leica ASP 6025S tissue processer, embedded in paraffin (Leica Paraplast) using a HistoCore Arcadia H. Tissues are then sectioned on a microtome (Leica, RM2255) at 5 µm onto positively charged slides (Fisher Scientific, 12-550-15).

28 Prior to Hematoxylin & eosin (H&E) and Alcian Blue-periodic acid-Schiff (AB-PAS) staining, slides are air dried overnight and baked for 30 mins at 60°C. H&E stains are performed using the Autostainer XL from Leica Biosystems. The slides are stained with Hematoxylin (Epredia, 7211) for 2 mins and Eosin -Y (Epredia, 7111) for 1 min. Clarifier 2 (7402) and Bluing (7111) solutions from Epredia are used to differentiate the reaction. After staining, slides are then dehydrated in graded ethanol, ending in xylene, and coverslipped with Cytoseal 60 (Epredia, 83104).

29 AB-PAS stains are also performed using the Autostainer XL from Leica Biosystems. The slides were stained with Alcian Blue (Anatech, LTD, 867) for 10 mins, immersed in Periodic Acid (ThermoFisher Scientific, A223-100) for 5 mins, rinsed in water, then transferred to Schiff reagent (Fisher Scientific, SS32-500) for 30 mins followed by a Sulfurous rinse for 1 min, and finally washed in running tap water for 10 mins. After staining, slides were then dehydrated and coverslipped with Cytoseal 60 (Epredia, 83104).

Recording and Analysis of Results

30 Complete the worksheet and update the virtual freezer inventory.

31 Correctly store photographs of the grossing process in the database.

Figure 1

32 **Figure 1. Dissection of distinct left upper lung regions. A.** Left lung lobe (Lateral view (Ai) and internal view (Aii)). **B.** Dissection of extrapulmonary bronchus (Region 1). **C.** 3rd generation bronchus isolated from the surrounding parenchymal tissue (Region 2). **D.** Dissection of bronchioles (~2 mm in diameter) (Region 3). PA: Pulmonary artery, Br: Bronchiole. **E.** Dissection of bronchioles (< 1 mm in diameter) (Region 4). **F.** Dissected alveolar tissue with pleura (Region 5).