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618.1_Lung MAP HTC Agarose Inflation of Non-fixed Human Lung Tissue, includes CMC embedding.

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We use this protocol and it's working

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Abstract

This protocol was developed by the Lung MAP HTC - BioRepository for Investigation of Neonatal Diseases of the Lung (BRINDL)

Its purpose and scope is to agarose inflate all or a portion of a lung lobe for later potential use in several preservation or experimental methods. These include five potential downstream Processes of Agarose Inflated Lung:

- Formalin Fixed Paraffin Embedded
- Paraformaldehyde Fixed Sucrose Protected OCT Frozen
- Un-Fixed OCT Embedded Frozen
- Un-Fixed CMC Embedded Frozen
- Precision Cut Lung Slices (PCLS)

Cell culture suitable agarose (see Materials) is prepared in DMEM cell culture media for potential metabolic support of the agarose inflated tissue. The protocol is written for human lung lobes but can be scaled to organs of other species.



Guidelines

At time of writing, personnel will adhere to safe work processes all consistent with recommended PPE for COVID-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 designate lab space that is covered by annually updated IBC approved protocol. All institutional biosafety measures are followed in any manipulation of these human tissues. In the post COVID era, procedure was reverted back to standard Biosafety level 2 procedures required for work with human tissues and fluids.

Materials

- a. Worksheet 603.A HTC_Whole_or_Partial_Lung_Processing
- b. Biosafety surface and environment for manipulation of lung
- c. Grossing Station, Biosafety Cabinet
- d. 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 work
- e. Large Water Bath
- f. Biohazard Disposal Bag
- g. Res Sharps Container
- h. Tissue mega-cassettes (optional) and uni-cassettes
- i. Plastic Cryomolds
- j. 100% OCT (Tissue Tek)
- k. 5% (w/v) Carboxymethylcellulose (CMC) in DEPC treated water
- l. 2 x Flat ice bucket partially filled with dry ice pellets
- m. Flat metal pan
- n. 100% ethanol or isobutene to create dry ice bath
- o. Aluminum foil and labeling tape or freezer storage bags
- p. Small biohazard stickers
- q. Freezerbondz Labels and Printer
- r. Rulers – metal 18 inch (45cm) and 12 inch (30.5cm)
- s. Scalpels and Trimming Blades with Handles; Forceps; Other Dissection Equip
- t. Labeling pencil
- u. Agarose, low gelling temperature Sigma A9045.
- v. 2% (w/v) Agarose in Gibco Phenol-Free DMEM
- w. Gibco Phenol-Free DMEM
- x. Hanks' Balanced Salt Solution (HBSS)
- y. Small and Medium Gauze Pads – multi-pack
- z. Standard Balance and Weigh Boats; Medium and Large
- aa. 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 3e/= 4.0cuffed
- bb. IV extension set tubing with clamp
- cc. 20ml syringe barrels for inflations
- dd. Needle free suture material
- ee. Bleach and/or Oxivir Tb
- ff. Wet Ice

Reagent Preparation

1 **2% (w/v) Agarose in DMEM**

Cell culture suitable agarose (see Materials) is prepared in DMEM cell culture media for potential metabolic support of the agarose inflated tissue. The amount of agarose prepared will depend on the size of the lung lobe being inflated. Typical volumes are between 200 mL – 500 mL.

As an example, for a lung requiring 200 mL of 2% agarose, 4g of low gelling-point agarose is added to a sterile glass bottle and then enough DMEM is added to bring the volume up to 200 mL. This solution can be heated in a microwave in pulses, being careful not to boil over the beaker, until the agarose has melted into the DMEM. Place the bottle in a clean heated (37°C) water bath to keep the agarose ABOVE 37°C and BELOW 40°C to respectively prevent premature gelling of the agarose and cellular damage when instilled into the lung.

Warm Hank's Balanced Salt Solution

In a clean histology water bath, heat to 37°C a sufficient volume of HBSS to cover the agarose inflated lung. Do not warm to above 39-40°C. This is done to prevent the agarose from hardening too quickly upon instillation into cold lung tissue, higher temperatures however may damage the lung tissue. Also prepare a container of ice cold HBSS to enhance agarose solidification in the lung after instillation.

Agarose Inflation

- 2 Select lung lobe to be inflated with agarose. If only a portion of a lobe is to be inflated, place a clamp across the lobe to prevent agarose entering tissue not to be inflated. Keep large airway used for inflation on necessary inflation side of clamp. This works well for the left upper lobe.
- 3 Inflation procedure should be accomplished in the warmed water bath with the operators taking appropriate biosafety precautions appropriate for work with human tissues.
- 4 Keep lobe chilled in transplant buffer on wet ice until ready to begin inflation procedure.
- 5 From beginning of inflation procedure, keep the surface of the lobe/tissue moist at all times and warmed in 37C water bath (filled with HBSS) throughout the duration of inflation procedure.
- 6 Cannulate the main bronchus, identified by its cartilage containing wall, with an endotracheal tube or 16 or 18 gauge catheter. Tie in place firmly with suture material, or

zip tie, placed so can be tightened around airway when cannula removed. Place the tube as deeply as possible to better secure it but not past a branch point of the airway. If two bronchi, due to branch-point, are apparent, each can be cannulated. Tying in of the catheters is the "trickiest" part of the entire procedure and is most easily done by two people.

- 7 Once catheter(s) are secured, place the lobe into 37°C HBSS filled water bath for 5 minutes to allow the lobe to warm. Make sure agarose solution (2% w/v low gelling temperature agarose in Phenol-Free DMEM, made up fresh on day of use) has also been warming in 37°C water bath. Prepare also a 20mL syringe in a 50mL conical.
- 8 When 5 minutes have elapsed, keeping the lobe in the warm HBSS, fill your syringe with the prepared 2% agarose and connect this to the end of the endotracheal tube. Slowly apply pressure to begin inflation, refilling the syringe with warm agarose as needed. Be sure to keep the 2% agarose solution at 37°C as much as possible, move quickly and gently. Repeat until lobe is moderately firm by inflation, avoid over filling and distending the lung. While moderate firmness of the inflated lung is desired, take care not to over inflate the tissue as the alveolar septae can be significantly stretched and thinned with over inflation.
It is best to have two people working on the inflation, having one person hold the lobe and catheter and the other filling and injecting the agarose solution from the syringe.
- 9 Once the lobe is fully inflated with agarose solution, have one person remove the catheter from the bronchus and the other pull the suture or zip-tie tight. If a clamp was used, leave this in place. Immediately place lobe in specimen container filled with ice cold HBSS for at least 45 minutes until agarose solution is completely solidified. Place the lobe into ice-cold HBSS (this container should also be on wet ice) for at least 40 minutes, until the lobe solidifies. Keep any floating portions of the lobe moist with gauze

Preparation for Use and Preservation of Agarous Inflated Lung

- 10 Photograph anterior and posterior sides before and after inflation/solidification and document all time points of inflation and volumes. Document time lobe went into water bath to warm, time inflation started, time inflation finished, time lobe went into ice cold HBSS, volume infused into lobe.
- 11 Remove the lobe from the ice-cold HBSS and slice and block the lobe, approximately 0.5-1cm thick, according to already established protocol (dx.doi.org/10.17504/protocols.io.biz7kf9n).
- 12 For preparaton of agarose inflated precision cut lung slices, see 623.2.HTC_Precision_Cut_Lung_Slices V.2
DOI: dx.doi.org/10.17504/protocols.io.4r3l2o26xv1y/v2
- 13 For long-term frozen storage, section the slices further into blocks (~1×1 cm to 1×2 cm 0.5-1 cm thick blocks. Photograph resulting tissue sections as a map of the stored



blocks.

Place the tissue blocks into labeled cryomolds, add additional 5% CMC to cover tissue and fill mold. Take care not to introduce bubbles in CMC. For standardization, orient tissue with lower cut side of slice facing down.

OCT can be used as an alternative to CMC. CMC is more compatible with mass spectroscopy based assays though somewhat more difficult to work with (melts rapidly and fragments more easily than OCT when sectioned frozen).

- 14 Place cryomolds containing tissue in CMC or OCT filled mold on metal pan in dry-ice bath and allow to freeze, add additional CMC / OCT to completely cover tissue

Once CMC / OCT block is frozen, may wrap in labeled aluminum foil, transfer cryomold to dry ice bucket/Styrofoam until blocking completed and then collect the cryomolds into labeled bags in freezer boxes and store in -80°C freezer.

Analysis of Results

- 15 Complete Worksheet and/or BRINDL Database CRF
Correctly store photographs of blocking in BRINDL Database

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Acknowledgements

Relevant Additional Standard Operating Procedures

1. Formalin-Fixed Paraffin Embedded (Following LungMAP SOP 616.01HTCBSL2+*Formalin Fixed Paraffin Embedded Tissue*)
2. Paraformaldehyde Fixed Sucrose Protected OCT Frozen (Following LungMAP SOP 617.1.HTCBSL2+Non-Inflated *Fresh FrozenTissue* BSL2+)
3. Non-Fixed OCT Embedded (Following LungMAP SOP 617.1.HTCBSL2+Non-Inflated*FreshFrozenTissue*BSL2+)
4. Non-Fixed CMC Embedded (Following LungMAP SOP 617.1.HTCBSL2+Non-Inflated*FreshFrozenTissue*BSL2+)
5. Precision Cut Lung Slices (Following LungMAP SOP 623.2.HTC_Precision_Cut_Lung_Slices V.2

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Revision History

Version | Changed By | Purpose | Change Date:

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618.01 | Rogers, L | New SOP for Agarose Inflation | 02162021

618.1 | Pryhuber | Finalized to Version 1 | 101122