# Standardised Operating Procedure

# Lung Biopsy

chILDRANZ 2020

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# Proposed Best Practice Checklsit

## Surgical Lung Biopsy

### The role of Surgical Lung Biopsy

A lung biopsy is carried out in chILD when the diagnosis is unclear and/or the clinical course is unexpected. It is strongly recommended that biopsy is preceded by a Multidisciplinary Team (MDT) meeting between physician, surgeon, radiologist and pathologist so all are fully informed to ensure that the appropriate area is biopsied and tissue samples are appropriately handled for relevant tests to cover the differential diagnoses.

### Risk-Benefits:

Transbronchial biopsy is less invasive but diagnostic yield is significantly reduced as the sample is frequently not representative being smaller and comprising only peripheral acinar tissue together with peri-bronchial elements. In addition to the risks inherent of thoracic surgery, wedge lung biopsy in a child with marked respiratory compromise may result in inability to wean from the ventilator post-surgery, while a trial of treatment, if successful, may avoid ventilation but post-treatment biopsy has a reduced diagnostic yield. Diagnostic yield is reduced in the late "end" stages of lung disease.

### Biopsy Procedure:

Area(s) to be biopsied agreed by MDT to include involved lung tissue +/- other area(s) that differ for uncertain reasons e.g. possible variation in activity/infection. Skin biopsy ought to be taken at the time for fibroblast cell line/DNA extraction/storage.

A wedge of lung at least 10x10x10mm should be sampled. 10 mm depth is necessary to include the terminal bronchiole area and associated vessels. The tip of lobes and the lingula should be avoided. The wedge should be sent, immediately, sterile and fresh, to the Anatomical Pathology laboratory for further handling.

## Anatomical Pathology Procedure:

The wedge biopsy is measured and the staple line removed sparingly. Sampling should be guided by MDT meeting discussion but will include:

a) Microbiology sample: small tissue slice (or can use the staple-line) sent fresh.

b) Electron Microscopy: 1mm thick tissue slice, in EM fixative (Appendix 1) with instructions to the EM laboratory to process to preserve glycogen. (Sample in fixative in <15 mins if possible.)
   - Abnormally structured lamellar bodies can be observed in surfactant deficiencies
   - Glycogen in PIG,
   - Storage material can be characteristic in some inborn errors of metabolism

c) Frozen Sample: Small sample snap frozen for: RNA studies / immunofluorescence, storage. (Appendices 2 & 3)

After sampling the wedge should be gently inflation fixed using standard formalin fixative via a fine needle then immersed in fixative. After a few hours it can then be sliced, base to apex, and processed to multiple paraffin blocks. Sections (5 micron) are then cut and stained with H&E for basic architecture with additional stains as required:

- Elastic Van Gieson: - collagen and elastin,
- Perls stain: haemosiderin / iron
- PAS / PAS-D: mucin, glycogen, pulmonary alveolar proteinosis.
- Ziehl-Neelsen, Grocott, PAS: infectious agents
- Immunohistochemistry: for identification of tumours, infections, specific cell types eg Langerhans cells (S-100, CD1a), vessels (CD34), neuroendocrine cells in NEHI (Bombesin).

## Surgical Pathology Report:

The biopsy report should be integrated with the clinical and radiological findings and built around the MDT commentary – providing comment on the adequacy / likely representative nature of the specimen and likelihood of the findings explaining the clinical and radiological features. Interpretation requires the expertise of a paediatric pathologist with experience in chILD which can be referred for an additional opinion, particularly in challenging cases. The pattern of morphological changes forms the basis of the differential diagnoses within the classification for paediatric diffuse parenchymal lung disease (Detering, 2019).

## Autopsy Specimen and Explanted Lungs:

All **explanted lungs** from diseased children, as well as unused corresponding donor lung should be immediately processed essentially covering the similar diagnostic pathways:

After samples are taken for EM, microbiology, frozen samples as above, then each lung should be inflation fixed with formalin using a fixation pressure of 20cm$H_2O$. If this is not possible then portions of the lung should be gently inflation fixed by formalin injection using a fine needle taking care not to overexpand. Multiple representative areas (selected as per guidance from the MDT meeting) should be sampled in 1.5 x 1.0 x 0.3 cm sections, processed to paraffin blocks, each labelled uniquely with the location within the lung from which the tissue was obtained and the case number.


## Acknowledgement:

European Management Platform for Childhood Interstitial Lung Diseases

Australian Genomics Health Alliance


## Further Support:

Please contact your local pathologist/s and surgeon/s for further advice pertaining to your site.

## References:


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Deutsch, G.H., *et al*. (2007). Diffuse lung disease in young children: application of a novel classification scheme. *Am. J. Respir. Crit. Care Med, 176*(11): p. 1120-8.

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El-Reshaid, W., K. El-Reshaid, and J. Madda. (2005). Postmortem biopsies: the experience in Kuwait. *Med Princ Pract, 14*(3): p. 173-6.

Langston, C., *et al*. (2006). A Protocol for the Handling of Tissue Obtained by Operative Lung Biopsy: Recommendations of the chILD Pathology Co-operative Group. *Pediatr. Dev. Pathol, 9*(3): p.173-80.

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## Appendix:

APPENDIX 1:  Glutaraldehyde Fixation Solution for Biopsies

**Chemicals:** Aqua ad injectabilia (Braun, Melsungen, Deutschland); Hepes (Sigma,H3375), paraformaldehyde (Merck), glutaraldehyde (Sigma,G6257).

**Protocol:**

1. 0,4 M Hepes (238,3 g/mol:  95,32g/L or 9,532g/100ml = 0,4 Mol). Adjust pH to 7,4.
2. Heat 45 ml H2O at 70°C, add 4g paraformaldehyde. Add appr. Xxx µl NaOH, or until solution turns clear. After cooling down of the solution the pH needs to be adjusted to pH of 7,4; add 0,4M Hepes to a final volume of 100ml.
3. Add glutaraldehyde to a final concentration of 0,1% (400µl).

**Note:** The buffer is stable for 4 weeks at 4°C or 1 year at –20°C, in this case prepare smaller aliquots.

APPENDIX 2.  Tissue for RNA, DNA and protein analysis

- Use RNA*later*® Solution with **fresh tissue only**; do not freeze tissues before immersion in RNA*later*® Solution.
- To ensure rapid and reliable stabilization of RNA even in the inner parts of solid tissues, the sample must be cut into slices **less than 0.5 cm thick** before immersion in RNA*later*® Solution
- The slices can be any convenient size, provided one dimension of the sample is <0.5cm.
- Place the fresh tissue in 5–10 volumes of RNA*later*® Solution (approx. 10µl RNA*later*® per 1mg tissue).
- Send immediately at ambient temperature to central biobank for further processing and long-term storage.

APPENDIX 3. Tissue for frozen section processing, immunohistology, RNA analysis, biochemical tests

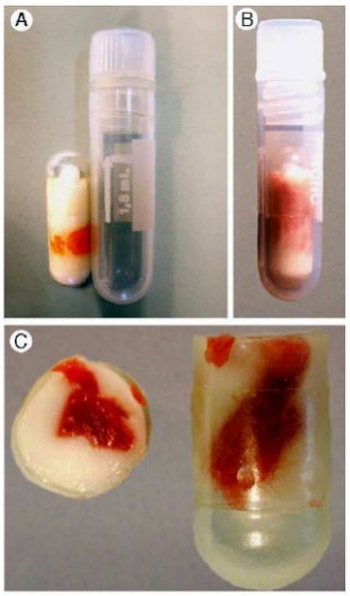


**Fig. 2** Capsule-freeze method of tissue storage. A, Cellulose capsule filled with tissue and frozen OCT next to a cryovial. B, The capsule fits easily within the cryovial for convenient storage. C, When needed, slices may be cut from the capsule for histological or other studies. The remaining tissue remains safely embedded within the OCT capsule.

- Maintain sterile conditions wearing gloves and using surgical scissors or scalpel and forceps.
- Add 1-2 drops of cryomatrix (Tissue Tek O.C.T Compound, Sakura, Ordering No. 4583) into cellulose capsules (Ordering No. 001033-55, Küppers-Primax GmbH, Troisdorf, Germany). Cryomatrix is a medium containing Optimal cutting medium (OCT).
- Place the tissue samples in the cellulose capsules, cover it with another 3-5 drops of cryomatrix; avoid formation of bubbles.
- Close the capsule and place into liquid nitrogen. After freezing, transfer capsule into a cryovial and store it in liquid nitrogen.
- Transfer from liquid nitrogen (if intermediate storage is necessary place into -80°C freezer) on dry ice and ship to the biobank for long-term storage.
- Always process several small pieces (appr. 3 x 3 x 5 mm).
- Long term storage is possible at -70° C or -196° C [2].
- After freezing the tissue into liquid nitrogen, PCR and biochemistry can still be performed. Histopathology will no longer be possible.

**Problems:** Artefacts associated with the capsule-freeze technique.