Surgical Lung Biopsy Protocols

Indications

- Unclear chronic or rapidly progressing lung diseases
- Ideally should be performed prior to commencing treatment

Relative contra-indications

- High risk due to advanced respiratory failure, other organ complication, high dose steroid or immunosuppressive therapy
- A child who is close to being ventilated is a particular diagnostic dilemma – the biopsy may mean the child remains ventilated after the procedure, whereas empirical treatment may not be indicated or missed without proper diagnosis
- Severe end-stage lung disease

Biopsies

- Biopsy should only be performed in centres capable of processing the biopsy correctly
- Surgical lung biopsy (open or video assisted thoracoscopic procedure) is the method of choice
- Biopsy sites are targeted pre-operatively through HRCT correlation to ensure appropriate areas are sampled
- Always take tissue from two different parts of the lung (e.g. upper lobe and middle lobe)
- Biopsy should yield a sample of at least 20x15x10mm. The tip of the lobe should be avoided.
- If an infection is assumed a separate specimen should be sent fresh and directly for microbiological study. If tissue is in short supply and a staple line is present, then the tissue attached to the staple line can be used. Alternatively swabs from surface and cut edges may be taken.
- We shake closed vial with biopsy pieces vigorously for 15 seconds to expand tissue (We do not inflate the tissue with formalin as this can cause artefact that mimics lymphangiectasia and wash out alveolar contents)

Important: Electron microscopy (EMI)

- Electron microscopy is very important for the diagnosis of some conditions and should be collected in all paediatric patients. Not having them is a real loss of potential for the diagnostic approach.
- Each lung biopsy specimen should be placed in in 2.5% Glutaraldehyde or Glutaraldehyde buffer and sent for correct embedding guaranteeing conservation of the lamellar bodies
- For assessment of paediatric interstitial lung diseases (e.g. suspected lamellar body deficiency) it is mandatory to use an elaborated prefixation before embedding of the specimen for Epoxid blocks are done
- We are offering this procedure within the lung-Registry free of charge .

1. Materials

1. 4% Formalin

- Commercially available

2. 2,5% Glutaraldehyd (usage of Glutaraldehyde in 0.1 M Na Cacodylat if available is superior)

- Commercially available
- Sent immediately for preparation of Epoxid-Blocks (needs to be done within days)

3. RNA*later*®

- Commercially available
- Ambion
- Ordering No. AM7020
- Store at room temperature

4. Liquid Nitrogen

- Commercially available

4.1. OCT (=Optimal cutting medium)

- Commercially available
- Tissue-Tek® O.C.T.™ Compound, Sakura
- Ordering No. 4583

4.2. Cellulose capsules

- Commercially available
- Küppers-Primax GmbH, Troisdorf, Germany
- Ordering No. 001033-55

4.3. CryoTube (1,8ml)

- Commercially available
- NUNC® Cryotube
- Ordering No. 363401

5. RPMI-Medium

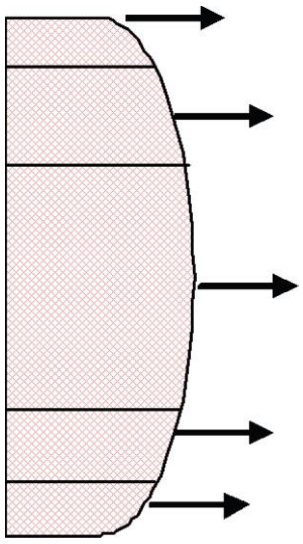
- Commercially available
- Store at room temperature

2. Preparation of the biopsy specimen

There are five principal pathways each lung biopsy specimen should go through:

1. Fixation for conventional histology and wax block (Formalin):
65% of tissue material (one piece of 10 x 10 x 10 mm)
2. Fixation for electron microscopy (in Glutaraldehyde):
5% of tissue material (5 pieces of 1 x 1 x 1 mm)
3. RNA, DNA and protein analysis (RNA*later*®):
5% of tissue material (one piece 3 x 3 x 2 mm)
4. Freezing in liquid Nitrogen for section processing, immunohistology, RNA analysis and biochemical tests (OCT and cellulose capsules):
20% of tissue material (one piece of 3 x 3 x 5 mm)
5. Cultivation of fibroblasts (RPMI):
5% of tissue material (one piece of 3 x 3 x 2 mm**)**

If 4. and 5. Are not done, enlarge the pieces of 1. and 3.



5% of tissue material (5 pieces of 1 x 1 x 1 mm) for EMI

20% of tissue material (one piece of 3 x 3 x 5 mm) for freezing

65% of tissue material (one piece of 10 x 10 x 10 mm) for formalin

5% of tissue material (one piece of 3 x 3 x 2 mm): for RPMI

5% of tissue material (one piece 3 x 3 x 2 mm): for RNA*later*®

3. Fixation of the biopsy specimen

1. Fixation for conventional histology and wax block (Formalin):

65% of tissue material (one piece of 10 x 10 x 10 mm)

- Tissue for conventional histology
- Fix tissue blocks in 4% formalin
- Local pathology will produce slides and embed tissue into paraffin (= wax block) as storage in formalin is only possible for a short period of time.
- Sent wax block at ambient temperature to central Biobank for further distribution to the reference pathologist

2. Fixation for electron microscopy (Glutaraldehyde)

5% of tissue material (5 pieces of 1 x 1 x 1 mm)

- Tissue for electron microscopy
- Cut the tissue into five pieces and placed in unfrozen 2.5% glutaraldehyde buffer
- Sent Glutaraldehyde at ambient temperature to central Biobank

3. RNA, DNA and protein analysis (RNA*later*®):

5% of tissue material (one piece 3 x 3 x 2 mm)

- For rapid and reliable stabilization of RNA even in the inner parts of solid tissues
- Place the fresh tissue in 5–10 volumes of RNA*later*® Solution (approx. 10 µl RNA*later*® per 1 mg tissue)
- Send at ambient temperature to central biobank for further processing and long term storage

4. Freezing in liquid Nitrogen for immunohistology (OCT, cellulose capsules and CryoTube):

20% of tissue material (one piece of 3 x 3 x 5 mm)

- Tissue for long term storage, immunohistology, RNA analysis and biochemical tests
- Add 2 drops OCT into cellulose capsules and place the tissue in the cellulose capsule. Cover it with another 2 drops of OCT, close the capsule and put it into a CryoTube (1,8ml). Then place it into liquid nitrogen
- Sent CryoTube on dry to central biobank for long-term storage

5. Cultivation of fibroblasts (RPMI-Medium):

5% of tissue material (5 pieces of 1 x 1 x 1 mm)

- For cultivation of fibroblasts and long-term storage
- Put the tissue in RPMI
- Sent RPMI-Medium at ambient temperature to central Biobank

Identification of subject (only if collection number was not entered into the data base): Pat-ID / Add-ID (SecuTrial) Name (if not pseudonomized):	**Identification of samples** by Collection number (#) paste label here: 45678 **If label not available indicate collection number:**

Sent to	**From**
Ms. Schams / Ms. Wesselak Room KO.10 Forschungszentrum Kubus Dr. von Hauner Children's Clinic Lindwurmstr. 2a 80337 Munich Germany	Name: Institute/Address: Phone number:

Study number:	**Study name:**

Visit number or visit date:	Collection # of the corresponding visit 1 of the patient

Date – Sample(s) taken	**Date – Sample(s) shipped**	**Shipping condition**

Number of tubes	Shipped samples from index patient	Number of tubes	Shipped samples from relatives Identify on TUBE !
	EDTA blood child		EDTA blood mother
	Tempus blood		EDTA blood father
	Biopsy in RNAlater solution		EDTA blood other relative, please specify who:
	Biopsy as wax block (for pathology)		
	Biopsy sildes (for pathology)		
	Biopsy in formalin (for pathology)		
	Biopsy in glutaraldehyde buffer/glutaraldehyde solution (for EMI)		
	Biopsy in DMEM etc for cell culture		
	Buccal swabs / saliva samples		
	Other, please specify		
	BAL supernatant		
	BAL cells		
	Serum		
	Citrate plasma		
	EDTA plasma		
	Biopsy, frozen in liquid nitrogen **(No histology possible!)**		
	Other, please specify		Other, please specify:

Ship at ambient temperature!

Ship frozen on dry ice!!

In case of enquiries please contact
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