



## A: General information of sample taking

General information concerning the sample taking can be found at the following link:

[http://www.klinikum.uni-muenchen.de/Child-EU/en/care/best\\_practice\\_checklist/index.html](http://www.klinikum.uni-muenchen.de/Child-EU/en/care/best_practice_checklist/index.html)

## B: General note on labeling the specimens

For labeling all specimens use the following form which is automatically sent to you. If you need more forms or did not receive a form please do not hesitate to contact us:  
Email: [chILD-EU.register@med.uni-muenchen.de](mailto:chILD-EU.register@med.uni-muenchen.de)

Fig. 1. Biomaterial and shipping conditions

**Biomaterials and shipping conditions**

Please ship the following samples at **ambient temperature** directly to the Biobank:

- EDTA Blood** (required for inclusion in chILD-EU register):
  - Take one vial from child (5 ml) / mother (10 ml) / father (10 ml) for shipping to the Biobank.
- Blood in Tempus tube for RNA isolation:**
  - Take one vial (3 ml) from the child for shipping to the biobank.

Other room-temperature material including but not limited to:

- Biopsy pieces in RNA/laser solution
- Biopsy pieces in glutaraldehyde buffer
- Biopsy in formalin
- Biopsy – wax block
- Biopsy – unstained slides
- Buccal swabs/saliva samples

Please label other materials with a blank label indicating sample type and relationship to patient!

**Blank labels child (specify sample type):** child 1-011-01, mother 1-012-01, father 1-013-01, Tempus child 1-021-01

**Blank labels (i.e. relatives):** child 1, child 1, child 1, child 1

(mark labels with permanent pen)

**Material that needs to be shipped frozen:**  
Frozen samples can be collected and shipped in batch to the Biobank

- BAL
- Serum
- Citrate-Plasma
- Biopsy (N<sub>2</sub> snap frosted)
- Other materials (i.e. urine, buccal swabs/saliva samples)

Please label other materials with a blank label indicating sample type and relationship to patient!

**Blank labels child (specify sample type):** child 1, child 1, child 1, child 1

**Blank labels (i.e. relatives):** child 1, child 1, child 1, child 1

(mark labels with permanent pen)

SOPs for biomaterial processing can be found at [www.childeu.net/en/care/best\\_practice\\_checklist/index.html](http://www.childeu.net/en/care/best_practice_checklist/index.html).

Please do not forget to enter biomaterial IDs in the chILD-EU register database for the respective patient ID.

**Explanation of sample labeling system:**

Sample ID: i.e. 17-011-01

17 = sample set ID  
11 = child's blood  
01 = aliquot number

Paste sample set ID.

Fill in patients ID (found in the database)

Label the blood samples:  
Childs blood always has the number 11  
Mothers blood always has the number 12  
Fathers blood always has the number 13

Aliquot number



## **C: General note on transportation of the specimens**

1. EDTA blood, serum, Tempus blood, all material fixed for conventional histology, electron microscopy and tissue in RNA/ater® Solution can be sent at room temperature. Please send the blood samples from the child and parents together.
2. For liquid probes, the vials must be placed in a protective container for the transport of biological and medical material and wrapped in shipping foam (Fig. 2).

Fig. 2 Shipment of samples





3. Frozen material must be shipped on 3-5 kg dry ice which is durable for at least 48 h. Please use an adequate stable styrofoam-box. Acceptance of the samples is only possible from Monday to Thursday.
4. Fill in the sample shipping sheet (Fig. 3).
5. The shipment must be labeled “UN3373 biological sample” (Fig. 2) and send per courier service; in France telephone number of the consignor is also required.
6. Please send an email with the tracking number to the following address when the samples are on the way:

Email: [chILD-EU.register@med.uni-muenchen.de](mailto:chILD-EU.register@med.uni-muenchen.de)



Fig. 3 Sample shipping sheet


## Sample shipping sheet – Consultation –

Patient name \_\_\_\_\_

Birth date \_\_\_\_\_

If available, paste  
sample set label here



<b>Sent to</b> Mrs. Schams / Mrs. Wesselak Room KO.10  Forschungszentrum Kubus Dr. von Hauner Children's Clinic Lindwurmstr. 2a 80337 Munich Germany	<b>From</b> Name:  Institute/Address:   Phone number:
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<b>Study name/number</b>	

<b>Visit number</b>	<b>Sample set ID of the corresponding visit 1 of the patient</b>

<b>Date – Sample(s) taken</b>	<b>Date – Sample(s) shipped</b>	<b>Shipping condition</b>

Number of tubes	Shipped samples from index patient	Number of tubes	Shipped samples from relatives of index patient
	<input type="checkbox"/> EDTA blood child <input type="checkbox"/> Tempus blood <input type="checkbox"/> Biopsy in <u>RNAlater</u> solution <input type="checkbox"/> Biopsy in <u>glutaraldehyde buffer/glutaraldehyde solution</u>		<input type="checkbox"/> EDTA blood mother <input type="checkbox"/> EDTA blood father <input type="checkbox"/> EDTA blood other relative, please specify: _____
	<input type="checkbox"/> BAL supernatant <input type="checkbox"/> BAL cells <input type="checkbox"/> Serum <input type="checkbox"/> Citrate plasma <input type="checkbox"/> <u>Biopsy, frozen in liquid nitrogen</u> <input type="checkbox"/> Citrate plasma <input type="checkbox"/> <u>Biopsy, frozen in liquid nitrogen</u>		
	<input type="checkbox"/> other, please specify:		<input type="checkbox"/> other, please specify:

Please put  
sample set  
label here



## **D: Specimens shipped at room temperature**

### **1. EDTA blood**

**5 ml of the patient**

**5 -10 ml of each parent and every other affected relative**

Collection into EDTA tubes. Ship the same day or the next day in double protected vials to avoid spilling. In case EDTA blood is not available, you can also send other sample types (e.g. heparine, citrate, coagulated, blood cake).

The division into aliquots will be done by the biobank.

### **2. Tempus blood - stabilization of RNA in blood**

For this purpose Tempus-Vacurette (Life Technologies) will be used and provided upon request by the biobank (Fig. 4). Either use Vacurette withdrawal system or when using an alternative blood withdrawal system, transfer blood directly through a needle into the Tempus-Vacurette container.

- Draw at least 2,5 ml blood. Ideally the containers are filled to the maximum height = 3.5 ml of blood.
- Shake vigorously or vortex for 10 sec

The Tempus container can be shipped together with the EDTA blood at room temperature. The RNA in the blood will be stabilized by the Tempus solution. Ship the same day or the day after. The duration of transport must not last longer than 5 days.

Fig. 4 Tempus-Vacurette container



### **3. Serum**

(0,5-) 2 ml. For preparation leave the blood without centrifugation at room temperature for 20-30 min then centrifuge at 1300 g, 10 min at room temperature. Ship at room temperature with EDTA blood the same day or the next day, or immediately freeze at -20°C for shipment on dry ice later (see E below).

**Remark:** Shipping temperature depends on the required analysis: for stable analytes shipment can be at room temperature; for unstable analytes susceptible to degradation shipment must be on dry ice. Shipping on dry ice preferred if shipped together with BAL.



## **4. Lung tissue**

There are four principal pathways every lung biopsy should go through.

1. Submit majority of tissue into wax blocks (formalin)
2. Take 2-3 small pieces for electron microscopy (glutaraldehyde buffer)
3. Store some tissue:
  - (a) in solution to conserve RNA and proteins (e.g. RNAlater® Solution) or
  - (b) **frozen using the protocol below (see E, 3.(b)).**
4. If tissue amount is small the tissue in the staple line can be sent to local microbiology.

### **1. Formalin fixation for conventional histology (wax block)**

Tissue for conventional histology (About 70 to 80% of the tissue blocks will be formalin fixed for conventional histology)

- Fix tissue blocks in 4% formalin
- Local pathology will produce wax blocks and slides; the slides are read.
- The wax block will be sent to the Register for further distribution to the reference pathologist
- Alternatively the wax block may be shipped directly to the reference pathologist. Slides are produced, read and then sent together with the wax blocks to the collecting point at the Kids Lung Register.

*Long term storage:* Storage in formalin is only possible for a short period of time. Therefore embedding into paraffin (= wax block) is essential.

### **2. Glutaraldehyde fixation for electron microscopy (Epoxyd block)**

Tissue for electron microscopy

- Three pieces per biopsy site, of about 2x2 mm, are placed in glutaraldehyde buffer (recipe below). The buffer needs to be stored frozen (-20°C) until used.
- Ship to biobank for embedding into epoxide

*Long term storage:* Material for the electron microscopy cannot be stored in glutaraldehyde and must be embedded in epoxide blocks. Alternatively, one part of the sample can be stored in glutaraldehyde buffer (like the one described above), while the other part is embedded into epoxide (service of the KLR).

#### 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 H<sub>2</sub>O at 70°C, add 4 g 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 0,4 M 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.

### **3. (a) RNAlater® Tissue Collection**

#### Tissue for RNA, DNA and protein analysis

- Use RNAlater® Solution with fresh tissue only; do not freeze tissues before immersion in RNAlater® 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 RNAlater® Solution. The slices can be any convenient size, provided one dimension of the sample is <0.5 cm.
- Place the fresh tissue in 5–10 volumes of RNAlater® Solution (approx. 10 µl RNAlater® per 1 mg tissue)
- Send immediately at ambient temperature to central biobank for further processing and long term storage

### **4. Detection of pathogens (by local microbiology, if desired)**

#### Material for microbiology

- Obtain sterile microscope slides for Gram staining of the cut surface of a biopsy. Usually three slides air dried and three fixed with alcohol.
- Additionally small pieces from the edges or left overs can be put in sterile saline for culture and PCR under sterile conditions.
- Close liaison with the microbiology laboratory prior to performing the biopsy is essential.



## **E: Specimens shipped frozen on dry ice**

### **1. BAL**

The recovered fluid should be kept at 4° C before analysis to optimize cell viability.

Centrifuge 180 g for 10 min

- Cells (in pellet) are used for cytopins. At least 10 slides per patient. Air dry and ship (at room temperature! Do never freeze) to Register. Rest of cells may be stored frozen and shipped on dry ice.
- Supernatant stored at -70°C until shipment on dry ice.

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### **2. Tracheal aspirate**

- Best done before any surfactant application.
- Last exogenous surfactant application at least 30 days ago.
- Try to collect as many tracheal aspirates (> 10) as possible over a couple of days until the last minute before the samples are shipped.
- Tracheal aspirates are collected in mucus traps which should directly be frozen in a -20°C freezer without further treatment.

### **3. (b) Lung tissue**

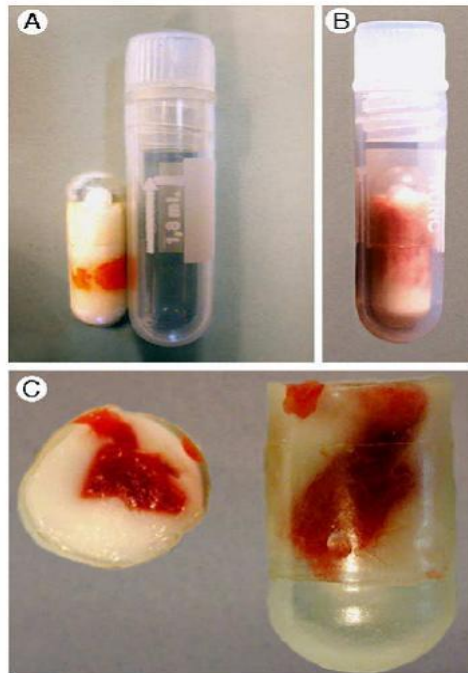
**Direct freezing of the tissue in OCT and liquid nitrogen**

Tissue for frozen section processing, immunohistology, RNA analysis, biochemical tests

- 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.
- 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.

\*If no Cryomatrix is available prefer placing sample into RNAlater solution (see D, 3. (a)) or freeze the tube directly in liquid N<sub>2</sub>

Fig. 5 Capsule freeze method of tissue storage



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.