



Biomaterial handling

A: Safest is to use our Sample shipping sheet: (see link above at the homepage)

Sample shipping sheet



Identification of subject
(only if collection number was not entered into the data base):

Pat-ID / Add-ID (Securial)

Name (if not pseudonymized):

Identification of samples

by **Collection number (#)** paste label here:

45678

If label not available indicate collection number:

Sent to Ms. Schams / Ms. Wesselak Room KO.10 Forschungszentrum Kubus Dr. von Hauner Children's Clinic Lindwurmstr. 2a 80337 Munich Germany	From 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 aHc:
	Biopsy as wax block (for pathology)		
	Biopsy slides (for pathology)		
	Biopsy in formalin (for pathology)		
	Biopsy in glutaraldehyde buffer/glutaraldehyde solution (for EM)		
	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:

In case of enquiries please contact
Ms. Andrea Schams: +49 89 4400 53715, chILD-EU.register@med.uni-muenchen.de



Ship at ambient temperature!



Ship frozen on dry ice!

B: Labeling the specimens

For samples to be sent pseudonymized great care has to be taken with the labeling, because if not clearly identified, the material cannot be used!

We use in our Biobank a **sample ID** (Sample identification number)

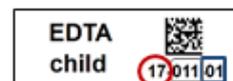
It consists of 4 sections (numbers: # stands for “number”). NOTE: Section 1 is mandatory!



- 1. Collection number** (= collection #) is unique for a patient and a visit. This means that it is different for each collection of samples done. The numbers are distributed by the register and if not available in a center must be asked for at chILD-EU.register@med.uni-muenchen.de. At least this number must be indicated on all samples shipped from one collection AND in the field "Collection number" in the register (see left). Otherwise it is not possible to link samples to a patient.
- 2. Type number** (= type #). If code is not at hand, just indicate in writing on tube.
- 3. Donor number** (= donor #). If code is not at hand, just indicate in writing on tube.
- 4. Aliquot number** (= aliquot #).

Explanation of sample labelling system ----- (# stands for "number"):

Sample ID: example: 17-011-01



17	01	1	01
Collection #	Type #	Donor #	Aliquot #
up to 5 digits	i.e. blood, biopsy, ...	(= indicator for index patient (=1), mother=2, father=3)	
Important and a must: as unique for patient and visit. Is the same for all samples from one collection	i.e. BAL 03		
	EDTA 01, ...		
	after choosing sample type and donor from a list, these digits will be filled in automatically by the database		

Some sheets with collection numbers and labels fitting for -80C tubes will be send to you on REQUEST in the following format (Fig 3):

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



Biomaterial labelling 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.

Paste collection number (#) label on top of the sample shipping sheet

EDTA blood child	mother	father	Tempus blood child
EDTA child 1-011-01	EDTA mother 1-012-01	EDTA father 1-013-01	Tempus child 1-021-01

Other room-temperature material, including but not limited to:

- Biopsy pieces in RNA/later 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):	Blank labels (i.e. relatives)
child 1	child 1

(mark labels with permanent pen)

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



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

- **BAL**
- **Serum**
- **Citrate-Plasma**
- **Biopsy (N₂ snap frosted)**
- **Other materials (i.e. urine, buccal swab)**

BAL	Serum	Citrate plasma	Biopsy (N₂-frosted)
BAL child 1-041-01	Serum child 1-081-01	Citrate child 1-091-01	Biopsy child 1-101-01

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

Blank labels child (specify sample type):	Blank labels (i.e. relatives)	Stick label on sample shipping sheet:
child 1	child 1	1

(mark labels with permanent pen)

SOPs for biomaterial processing can be found at www.childeu.net/en/care/best_practice_checklist/index.html.

=> Do not forget to enter collection # in the **child-EU** register database for the respective patient ID.

Explanation of sample labelling system (# stands for "number"):

Sample ID: example: 17-011-01

17-	01 1-	01
Collection # up to 5 digits Important and a must, as unique for patient and visit. Is the same for all samples from one collection	Type # Donor # I.e. blood, biopsy, ... (= Indicator for Index patient (=1), mother =2, father =3) I.e. BAL 03 EDTA 01, ... after choosing sample type and donor from a list, these digits will be filled in automatically by the database	Allquot #



C: Transportation of the specimens

1. EDTA blood, serum, Tempus blood, all material fixed for conventional histology, electron microscopy and tissue in RNA/ater® Solution **MUST** be sent at room temperature.
2. Vials must be placed in a protective container for the transport of biological and medical material and wrapped in shipping foam.



3. Frozen material must be shipped on 3-5 kg dry ice (durable for about 48 h). Please use a styrofoam-box. Acceptance of the samples is only possible from Monday to Thursday.
4. Fill in the sample shipping sheet
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



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. 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 (Epoxy 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 epoxy

Long term storage: Material for the electron microscopy cannot be stored in glutaraldehyde and must be embedded in epoxy 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 epoxy (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 H₂O 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 cytopspins. 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.

http://www.klinikum.uni-muenchen.de/Child-EU/en/care/best_practice_checklist/index.html

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 N₂

Fig. 5 Capsule freeze method of tissue storage

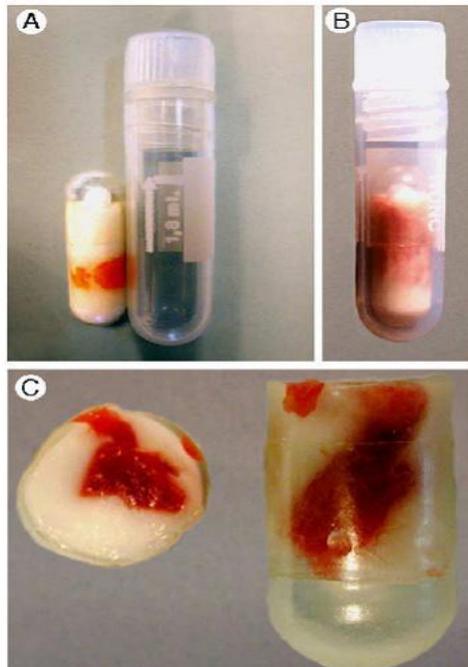


Fig. 2 Capsule-freeze method of tissue storage. A, Cellulose capsule filled with tissue and frozen OCT next to a cryovial. B, The capsule fits easily within the cryovial for convenient storage. C, Sliced, slices may be cut from the capsule for histological or other studies. The remaining tissue remains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