1. Sample Material

**Specimen collection**

- Bone marrow aspirate
- Peripheral blood
- Lumbar puncture: native cerebrospinal fluid, without anticoagulant
- Pleural effusion/ascites: native, without anticoagulant
- Trephine biopsy/tissue biopsy: place biopsy into NaCl 0.9 % with heparin (500 I.U./ml), no formalin
- Paraffin embedded sample material (only selected analyses can be performed based on DNA isolated from formalin-fixed paraffin embedded (FFPE) sample material)
- DNA
- cDNA
- RNA (shipment on dry ice)
- Cryopreserved cells

Unsuitable sample material: peripheral blood without anticoagulant, serum

2. Required sample volume

Depending on the test method, different volumes of sample material are required:

- **Chromosome analysis:** if possible 5-10 ml bone marrow aspirate, or in case of suspected CLL peripheral blood
- **FISH**: 2-3 ml bone marrow aspirate or peripheral blood are sufficient in case of normal cellularity; alternatively, prepared, non-fixed, unstained smears can be examined
- **Cytomorphology**: 6-8 unstained bone marrow smears and peripheral blood smears each, alternatively liquid sample material for preparation of smears
- **Molecular genetics**: preferably 5-10 ml bone marrow aspirate/peripheral blood/other sample material after consultation, 15-20 ml for follow-up examinations of peripheral blood
- **Immunophenotyping**: preferably 5-10 ml bone marrow aspirate/peripheral blood, 3-5 ml cerebrospinal fluid/pleural effusion/ascites

A total volume of 10 ml bone marrow is sufficient to perform all the tests described above.

### 3. Processing of samples

#### 3.1 Specification of anticoagulants

Please consider anticoagulant restrictions for each method of analysis:

- Chromosome analysis: heparin feasible; no EDTA, no citrate
- FISH: EDTA or heparin feasible
- Cytomorphology: EDTA or citrate feasible, no heparin
- PCR/mutation analysis/immunophenotyping: EDTA, heparin and citrate all feasible

#### 3.2 Filling the samples into a tube without anticoagulant

Bone marrow/peripheral blood
- Add an anticoagulant: heparin (500 I.E./ml), EDTA, citrate
Bone marrow touch preps/trephine biopsies (punctio sicca): for cytogenetics and molecular genetics, **no formalin**, use NaCl 0.9% with heparin (500 I.E./ml)

Other material: liquor/pleural effusion/ascites: native, without any anticoagulant

### 3.3 Labeling of tubes

Label all tubes with surname and first name, date of birth as well as collection date.

### 3.4 Requirements for referral of isolated DNA

We recommend sending the DNA undiluted. The concentration should not be below 20-25 ng/μl.

Please indicate the concentration of the DNA, the primary material (if necessary fixation) and if possible also the quantification method. In general, 1 μg DNA is required for the analyses of several genes. For example, 50 μl with a concentration of 20 ng/μl can be sent. Analysis of individual hotspots (e.g. FLT3-TKD) or our MPN panel (JAK2, CALR and MPL) can be performed with 600 ng of DNA.

### 4. Completing the order form

**Details on samples and analyses needed:**

- Mark the respective box for shipped sample material (bone marrow, peripheral blood, other sample material)
- State the number of shipped smears (separately for bone marrow and peripheral blood)
- State the date and time of withdrawal
- Tick the respective box for diagnostic sample or follow up sample
- Identify the study and study number (if applicable)
- Mark the respective box(es) for analyses to be ordered (cytomorphology, immunophenotyping, chromosome analysis, FISH, molecular genetics)
Patient details:
- surname, first name, date of birth, gender, address, insurance (labels can be used as well)

Details on laboratory parameters:
- WBC count, hemoglobin level, thrombocyte count, differential blood count (if available), further pathological results (if available)

Details on data specific to disease:
- diagnosis/suspected diagnosis
- course of therapy (e.g. medication administered so far, bone marrow transplantation with details on the donor’s gender, chemotherapies)

Details on client:
- identification of referring physician and institution (name, phone number, fax number, stamp)

Further details:
- the analyses to be ordered can be specified in more detail on the additional pages of our order form

Completing the Declaration of Informed Consent:
- date and patient’s signature of the consent form
- patient’s data (surname, first name, birth date, address)

Address the sample material to:

MLL/MLL Dx
P.O. Box 20 14 53
5. Packaging, labeling and transport of samples

5.1. Packaging

Most sample material being sent to our laboratory is listed as “sample material“ (according to paragraph 2.2.62.1.5.6. of the Recommendations on the Transport of Dangerous Goods by UNECE these are defined as sample material “with minimal risk of containing pathogens“).

The packaging of clinical samples has to be constructed in a way that it can withstand all forces during transport and prevent any release of the sample material.

- Packaging for exempt samples has to consist of three parts (composite packaging):
  a) waterproof primary container (e.g. monovette)
  b) waterproof secondary packaging
  c) sufficiently robust outer packaging (surface dimension of at least 100 x 100 mm)

The secondary packaging has to be put into the outer packaging along with adequate amount of padding material. Fluid sample material requires sufficient absorbing material between the tube and secondary packaging. This packaging roughly matches P650 and is often called „P650 light“.
- Packaging according to the standard P650 for infectious patient sample material and cultures of category B (UN-Nr. 3373)

These packages are equivalent to exempt samples (triplicate packaging). Packaging according to P650 requires either an inflexible/rigid secondary packaging or outer packaging. Additionally, either the primary container or the secondary packaging needs to withstand a pressure difference of 95 kPa. Further, the whole package needs to withstand a drop test from 1.2 m height without damages.

Shipment material can be ordered from our laboratory.

5.2 Labeling and documentation

For exempt human samples shipment has to be marked as „exempt human specimen“ in addition to indicating sender and recipient.

5.3 Transport and mailing

A maximum transportation time of 24 hours should be targeted. This will be warranted by a 24h express service (e. g. DHL, UPS, TNT and GO!) or other providers of express mail service. When sending sample material on Fridays please make sure to order “Saturday delivery”. Samples on Sundays will be accepted only after prior consultation and arrangement.

Please mind the differing transport modalities that apply to the respective transport firm. Please call us for advice on (+49-(0)89-990170).

When sending samples prior to a public holiday, please make sure you order „Public Holiday Delivery“. Be especially aware of holidays that are public only in certain federal states of Germany:

January 6th – Epiphany
Corpus Christi on the second Thursday after Whitsun
August 15th – Assumption of Mary
November 1st – All Saints’ Day.
6. Withdrawal of bone marrow and venous peripheral blood

6.1 Sampling of venous peripheral blood

The required collection tubes and cannulas are prepared and the collection tubes are labeled with the patient’s first and last name, collection date and date of birth before collection. The patient sits and puts his arm down, the tourniquet is applied. The area to be punctured is disinfected with disinfection spray. Wait approx. 30 seconds before performing the puncture. The vein is punctured with the cannula, the collection tube is attached and the blood aspirated, then the tourniquet is opened. The collection tube is removed at the end of the blood collection procedure and the needle is then pulled out (while simultaneously applying pressure to the puncture site with cellulose swabs). The swabs are folded, laid along the punctured vein and attached with a plaster strip under tension. The patient is instructed to press firmly on the injection point with two fingers for at least three minutes and not to bend the arm.

6.2 Bone marrow biopsy and aspiration

Samples should preferably be taken from the posterior iliac crest (spina iliaca posterior superior). The former sternal needle (Klima-Rosegger Needle) (to be used without the spacer for the pelvis) and a histology needle (so-called Jamshidi needle) are to be used for puncture.

6.2.1 Bone marrow biopsy

Bone marrow biopsy should be conducted before aspiration in order to acquire artifact-free tissue. The patient is placed in a prone position; after thorough disinfection and application of a sterile cover, the skin is anesthetized with at least 10 ml Ultracaine up to the periosteum of the spina iliaca posterior superior. The onset of satisfactory anesthesia will require at least five minutes. An 8-gage needle width is preferred, and the 11-gage needle has proved particularly effective for young patients with strong bones. A longer needle is also available particularly for obese patients. The needle is applied to the center of the posterior iliac crest; after removal of the mandrel, it is inserted through the cortex toward the usually quite easily palpable spina iliaca.
anterior. Biopsy lengths of up to 4 cm are possible and enable a representative assessment of the bone marrow. Moreover, a large biopsy permits an easier “twisting motion” and will ensure that the stamped bone marrow cylinder will be firmly enclosed in the hollow needle. If needed, biopsy touch preparations can be produced from the obtained cylinder. Depending on the intended use, the material is placed in physiological saline solution supplemented with heparin.

6.2.2 Bone marrow aspiration

After the biopsy has been obtained, the bone marrow aspirate is drawn. Klima and Rosegger puncture needles without arresting are most commonly used. Disposable needles should also be used here because of the sharper cut and for hygienic reasons. The bone marrow is punctured through the previous skin incision, at approximately 1 cm distance from the biopsy site and at an oblique angle to the biopsy direction. Before aspirating, the patient is made aware that a short-term pain will occur, which cannot be prevented even by careful local anaesthesia. Aspirate quickly and vigorously with a 10 ml syringe, up to the intended volume. If multiple tubes are withdrawn and all are taken from the same position, the last aspirates may have a different cell composition than the first aspirate, due to increasing dilution by peripheral blood. If aspiration is unsatisfactory, the position of the puncture needle must be changed by twisting or by puncturing again. In the case of punctio sicca, twisting the needle in the bone under continuous aspiration often allows for successful sample material withdrawal. If the puncture continues to be unsuccessful, the other side can be punctured additionally, or the anterior iliac crest can be punctured after appropriate anesthesia. After applying a bandage, the puncture site is compressed by lying on a sandbag. At the earliest 30 minutes later, the puncture site is checked for postinterventional bleeding.