Primary Sample Collection Manual

Labdia Labordiagnostik GmbH

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List of abbreviations

Abbreviation	Meaning
AIPF	Automatic immunofluorescence plus FISH
AML	Acute myeloid leukemia
B-ALL; T-ALL	B-cell and T-cell acute lymphoblastic leukemia
CML	Chronic myeloid leukemia
DEB	Diepoxybutane
EDTA	Ethylenediaminetetraacetic acid
EWOG	European Working Group of MDS and SAA in Childhood (EWOG-MDS/SAA)
FISH	Fluorescence in situ hybridization
HE	Hematoxylin and eosin staining
HLA	human leukocyte antigen
HR-NBL	High-risk neuroblastoma
IU	International unit (IU)
WiGeV	Vienna Healthcare Group
BM	Bone marrow
sol.	solution
min.	at least
ml	milliliter
µl	microliter
µm	micrometer
MRD	Minimal residual disease
NaCl	Sodium chloride
ng	nanogram
od.	or
PB	peripheral blood

Abbreviation	Meaning
PB-MNC	mononuclear cells from PB
PBS	phosphate-buffered saline
Phys.	physiological
PS	puncture site
qRT-PCR	Quantitative reverse transcription PCR
RNA	ribonucleic acid
RPMI	Roswell Park Memorial Institute Medium
RT	room temperature
SNP	single nucleotide polymorphism
STR-PCR	Short tandem repeats polymerase chain reaction
T-LBL	T-lymphoblastic lymphoma
WES	whole exome sequencing

1. Contact Details of Labdia Labordiagnostik GmbH

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Please refer to our website for the contact information of staff in the respective departments / specialist areas.
Website

2. General Information About the Organization

2.1 Opening Hours – Sample Reception

Monday – Friday	8:00 – 16:00
Saturday, Sunday and public holidays Sample reception exclusively for pediatric hematology study patients via St. Anna Children's Hospital	9:00 – 12:00

Outside these hours, prior notification by telephone is required.

Samples delivered outside the sample reception hours will be processed on the following business day. This may limit the interpretability of the results, which will be noted in the report.

2.2 Performance of Examinations

Examinations are generally performed at Labdia Labordiagnostik GmbH. If necessary or appropriate for technical or medical reasons, an examination may also be performed by another diagnostic laboratory in Austria or abroad.

2.3 Availability of Clinical Advice

Staff from the respective departments are available during opening hours to provide advice and consultation on examination requirements. Please refer to our website for contact information. website

Medical consultation regarding examination requests or the interpretation of examination results can be provided by the authorizing physician. Information is provided only to the referring physician or referring institution. Please refer to our website for contact information. website

2.4 Laboratory Policy on the Protection of Personal Information

To ensure confidentiality and the protection of personal information, Labdia Labordiagnostik GmbH complies with the applicable data protection regulations. Labdia Labordiagnostik GmbH has designated data protection officers who ensure and monitor compliance with data protection requirements (GDPR).

2.5 Feedback - Complaint Management

The contact form on the Labdia website can be used for feedback of any kind, except for patient-specific information. Feedback received is forwarded to the departments responsible and, in the case of complaints, appropriate action will be taken. Contact form

3. General information on the Primary Sample Collection Manual

3.1 Primary Sample Collection Manual

The following information is intended to support correct sample selection and collection. It also describes the optimal storage and transport conditions for the different sample types.

3.2 Pre-examination Processes

Please note that the quality of the results is significantly influenced by pre-examination processes. Deficiencies in the pre-examination phase cannot be compensated for during subsequent sample processing.

Key aspects of the pre-examination phase are:

- Selection of the correct sample type and anticoagulant
- Correct sample collection (collection technique) at a suitable collection site, including preparation of the patient
- Time of collection
- Volume of sample collected
- Storage of sample prior to shipment
- Transport conditions and duration

4. Sample Reception and Acceptance Criteria

To ensure optimal sample collection and processing, please observe our acceptance criteria.

Unambiguous labeling of all collected samples and the corresponding request form is required to ensure correct identification and is the responsibility of the referring institution. If no patient label is used, the sample container must be legibly labeled with patient-related data such as surname, first name and date of birth, as well as the sample type / sample identification (e.g. collection site).

4.1 Acceptance Criteria for the Request

- Identification of the referring institution
- Patient identification
- Specification of sample type
- Collection date and, where applicable, time of collection
- Suspected diagnosis / clinical question / indication
- Specification of the requested examination
- Any required forms (informed consent, declaration of cost coverage) must be completed, signed and submitted with the sample (see website)
- According to Section 69 of the Austrian Genetic Engineering Act, a genetic analysis may only be performed if written confirmation is available

4.2 Sample Acceptance Criteria

- Labeling: patient identification, sample type and, where applicable, collection site
- Selection of the appropriate sample material for the requested examination (see section 6)
- Sufficient volume for the requested examination (see section 6)
- Correct anticoagulant used (see section 6)
- Suitable transport conditions (see section 9)

If any of the acceptance criteria are not met, interpretability may be limited. A corresponding note will be included in the report.

> **Please note:**
> **Samples without unambiguous patient identification cannot be processed and analyzed!**

5. Sample Collection

Samples are collected exclusively by medically trained personnel in accordance with the internal requirements of the referring institution and the Austrian Medical Practitioners Act. Therefore, this Primary Sample Collection Manual does not describe sample collection or safe handling, including disposal.
Where applicable, study protocols and their sample requirements must be observed.

5.1 Bone Marrow, Peripheral Blood, Ascites, Pleural Fluid, Other Body Fluids

5.1.1 Sample tubes

Depending on the requested examination, samples may need to be collected with anticoagulants (see section 6). Please observe the manufacturer's instructions and study protocols.

Tubes with anticoagulant (ready to use)

- for BM, PB - select the anticoagulant according to the requirements of the respective examination

Tubes without anticoagulant

- BM, PB: addition of an anticoagulant required: heparin (500 IU/ml), EDTA or citrate
- BM cores/core biopsy (in case of dry tap): for molecular genetics, do not use formalin; use only 0.9% NaCl solution with heparin (500 IU/ml)
- Ascites and pleural fluid, other body fluids: transfer into tubes without anticoagulant

PAXgene tubes for neuroblastoma patients from Austria

- Within the HR-NBL-1 and HR-NBL-2 studies, an additional 0.5 ml bone marrow per puncture site and 2.0 ml peripheral blood should each be collected in a PAXgene Blood RNA tube and shipped without cooling.
- PAXgene tubes are available free of charge from St. Anna Children's Cancer Research, Tumor Biology Laboratory (tumorbiology@ccri.at, +43 1 40470 / 4055).

5.1.2 Venous peripheral blood

- Immediately after collection, mix the tube (with anticoagulant) thoroughly by inversion
- Anticoagulant dosage: amount and volume according to the request form and study protocol

5.1.3 Bone marrow biopsy and aspiration

- Immediately after collection, mix the tube / syringe (with anticoagulant) thoroughly by inversion
- Indicate the puncture site on the tube
- Number the syringes consecutively
- Anticoagulant: amount and volume according to the request form and study protocol
- In pediatric hemato-oncological neoplasms (B-ALL, T-ALL, AML), if it is not possible to obtain enough bone marrow and the peripheral blast percentage is >50%, peripheral blood is enough for flow cytometry, patient-specific qPCR for MRD, FISH, SNP array, and almost all accompanying research projects. If in doubt, please contact the study center and the performing laboratory.

5.2 BM Smears

- Immediately after aspiration, a drop of marrow blood is placed onto a slide near the labelled end. Using another slide (spreader slide), drag the slide back to the drop of blood and as soon as the drop of blood begins to spread along the edge of the spreader slide, promptly and smoothly drag the spreader slide along the length of the first slide.
- Bone marrow particles should be visible in the feathered edge
- Advantage of this method: → excellent visualization of individual cells → optimal cytological assessment
- Disadvantage of this method: rapid clotting of bone marrow aspirate → only a limited number of smears can be prepared
- If bone marrow particles are absent, cellularity and iron content cannot be reliably determined.

5.3 Urine and Stool

- Send urine and stool only in tightly sealable containers

5.4 Touch Imprints of Tumor Tissue

- Press a sterilely collected piece of tumor tissue several times onto an adhesive slide; do not smear
- Label the slide in pencil with patient identification and date
- Ship slides without cooling in an airtight shipping box
- At least 2 touch imprints per requested FISH examination; at least 10 touch imprints for SNP array

5.5 Tumor Tissue, Fresh or Frozen

- Place a sterilely collected piece of tumor tissue in a container with culture medium (RPMI medium) or 0.9% NaCl solution and ship on ice (refrigerated)
- Alternatively, the sterilely collected piece of tumor tissue may be snap-frozen in a container on dry ice and shipped with sufficient dry ice
- Indicate tumor cell content

5.6 Paraffin Block, Sections and Curls

- Paraffin blocks must be labeled with an identifier (histology number) and may be shipped without cooling - they will be returned after completion of the examination
- Alternatively: place 3-5 paraffin curls (20 µm section thickness) in a container and ship without cooling
- For FISH examinations: mount 3-5 µm sections on adhesive slides, label in pencil (patient identification) and ship at least 4 such slides per examination without cooling in a closed shipping box
- Always include an HE section with the relevant tumor area marked
- Indicate tumor cell content

6. Overview of Examinations with Requirements for Sample Material, Volume, Anticoagulants, Storage and Shipment

Examination		Suitable material	Required volume	Anticoagulants and special sample collection requirements	Storage until shipment	Shipment
Chromosome analysis	**Hematological malignancies**	BM	5 ml	heparin	RT	RT
		PB	5 ml	heparin	RT	RT
		Tumor, Pleural effusion, Ascites		fresh (tumor also in RPMI or 0.9% NaCl solution)	RT	RT
	Syndrome diagnostics	PB	5 ml #	heparin	RT	RT
	DEB test	PB	10 ml	heparin	RT	RT
Flow cytometry	**Immunological diagnostics MRD, follow-up (B-ALL; T-ALL; AML)**	BM syringe no. 1	at least 1 - 2 ml	heparin	4 °C - RT	RT
	Immunological diagnostics EWOG	BM	at least 1 - 2 ml	heparin	4 °C - RT	RT
	Immunological diagnostics Immunophenotyping	preferably BM	BM at least 1 - 2 ml	heparin	4 °C - RT	RT
		BM	at least 1 - 2 ml	heparin	4 °C - RT	RT
	Immunological diagnostics Suspected lymphoma	PB	2,5 ml	EDTA	4 °C - RT	RT
		CSF; Pleural effusion; Ascites: (at the request of the clinic and after consultation)	0.5 – 1 ml CSF, Pleural effusion, Ascites	no anticoagulant; CSF must be delivered within 2 hours	4 °C - RT	RT
	Detection of blast cells in peripheral blood (PB)	PB	2,5 ml	EDTA	4 °C - RT	RT

Examination		Suitable material	Required volume	Anticoagulants and special sample collection requirements	Storage until shipment	Shipment
	Dual-platform analysis; immune status (basic analysis)	BM	2 ml	heparin/ACD-A	4 °C	RT
		PB	2 ml	EDTA	4 °C	RT
		Stem cell apheresis products	2 ml	ACD-A	4 °C	RT or 4 °C
		CSF, Pleural effusion Ascites	0.5 – 1 ml CSF, Pleural effusion, Ascites	no anticoagulant; CSF must be delivered within 2 hours	4 °C	RT
	Single-platform analysis for quantitative CD34 determination	PB	2 ml	EDTA	4 °C	RT
		BM	2 ml	heparin/ACD-A	4 ° C	RT
		Stem cell apheresis products	2 ml	ACD-A	4 °C	RT or 4 °C
	Residual cell measurement in blood components (residual leukocytes)	PB	2 ml	EDTA	4 ° C	RT
		BM	2 ml	heparin/ACD-A	4 ° C	RT
		Platelet concentrates	2 ml	ACD-A	4 ° C	4° C
	Eosin-5'-maleimide bindings assay (EMA)	PB	2 ml	EDTA; State the date of the last red blood cell transfusion **Analysis Tue + Fri**	4 °C	RT
	Sorting of leukocyte populations for chimerism analysis. Follow-up after stem cell transplantation for hematological malignancies and immunodeficiencies. Only in combination with basic analysis	BM	at least 2 ml	heparin	4 °C	RT
		PB	at least 2 ml	EDTA	4 °C	RT
ELISPOT	**Secretion assay for detection of virus-specific T cells**	PB	4 ml	EDTA or heparin (detection only possible from 20 CD3/µl); **Sample reception Mon-Thu**	4 °C	RT

Examination		Suitable material	Required volume	Anticoagulants and special sample collection requirements	Storage until shipment	Shipment
Fluorescence in situ hybridization (FISH)	Hematological malignancies general	BM	2 - 5 ml	EDTA or heparin	RT	RT
		PB	2 – 5 ml	EDTA or heparin	RT	RT
	Plasma cell disorders	BM	2 - 5 ml	EDTA or heparin	RT	RT
	Burkitt lymphoma	BM	2 - 5 ml	EDTA or heparin	RT	RT
		BM smears	at least 2 piece(s)	unstained	RT	RT
	Lymphomas	BM	2 - 5 ml	EDTA or heparin	RT	RT
		Touch imprints	at least 4 piece(s)	-	RT	RT
		Pleural effusion; Ascites	2 - 5 ml	-	RT	RT
	Chimerism	BM	2 - 5 ml	EDTA	RT	RT
		PB	2 - 5 ml	EDTA	RT	RT
	Pediatric solid tumors	Fresh tumor	at least 1 piece(s)	in RPMI or 0.9% NaCl solution shipment within 24 hours	4 °C	4 °C
		Frozen tumor	at least 1 piece(s)	snap-frozen	-80 °C	dry ice
		Touch imprints	2 piece(s) per FISH analysis	-	RT	RT
		Paraffin block	at least 1 piece(s)	-	RT	RT
		Paraffin curls	5 x 50 µm curls	-	RT	RT
		Paraffin section	4 x 3 – 5 µm sections	-	RT	RT
	Syndrome diagnostics	PB	2 - 5 ml #	EDTA or heparin	RT	RT
HPLC	Therapeutic drug monitoring	PB	5 ml	EDTA	4 °C - RT	RT or 4 °C
	Hemoglobinopathy	PB	2 ml	EDTA	4 °C	RT

Examination		Suitable material	Required volume	Anticoagulants and special sample collection requirements	Storage until shipment	Shipment
Immunocytology (AIPF)	**MRD determination in neuroblastoma, rhabdomyosarcoma**	BM	at least 5 ml per puncture site	EDTA, additionally +0.5 ml per puncture site in PAXgene Blood RNA (for details see 5.1.1.) shipment within 24 h	4 °C	4 °C
		Apheresis product	at least 1 ml	shipment within 24 h	4 °C	4 °C
		other materials (effusion, CSF) after consultation	effusion 20 ml CSF at least 0.5 ml	shipment within 24 h	4 °C	4 °C
		Cytospin preparations from BM	2 puncture sites: at least 2 piece(s) 1 puncture site: 4 piece(s)	1.0 - 1.2 million MNCs per cytospin preparation	RT	RT
		If tumor cell infiltration is sufficient, genetic analyses (e.g. FISH) can be performed on this material.				
Mutation screening *	**Hematological malignancies**	BM	2 ml	EDTA or heparin	RT	RT
		PB	2 ml	EDTA	RT	RT
		DNA	after consultation (depending on the clinical question)	-	4 °C	4 °C
		Tumor	at least 1 piece(s)	in 0.9% NaCl solution	4 °C	RT
	Syndrome diagnostics	PB	2 ml	EDTA	RT	RT
		DNA	after consultation (depending on the clinical question)	-	4 °C	4 °C
		other materials (e.g. buccal mucosa, hair,etc.)	only after consultation	-	only after consultation	
	T-LBL	BM	5 ml	EDTA or heparin	RT	RT
		Fresh tumor	at least 1 piece(s)	in 0.9% NaCl solution	4 °C	RT
		Frozen tumor	at least 1 piece(s)	snap-frozen	-80 °C	dry ice
		Pleural effusion; Ascites	at least 5 million cells	-	RT	RT

Examination		Suitable material	Required volume	Anticoagulants and special sample collection requirements	Storage until shipment	Shipment
	pediatric solid tumors *	Fresh tumor	at least 1 piece(s)	in RPMI or 0.9% NaCl solution, shipment within 24 hours	4 °C	4 °C
		Frozen tumor	at least 1 piece(s)	snap-frozen	-80 °C	dry ice
		Touch imprints	at least 10 piece(s)	-	RT	RT
		Paraffin block	1 piece(s)	-	RT	RT
		Paraffin curls	5 x 50 µm curls	-	RT	RT
		Paraffin section	4 x 3 - 5 µm sections	-	RT	RT
		DNA	at least 10 µl (at least 50 ng/µl)	-	4 °C	4 °C
	BCR::ABL1 kinase domain*	PB	at least 3 ml (20 million cells)	EDTA	4 °C	RT
PCR	**Patient-specific qPCR for MRD determination (B-ALL; T-ALL; AML; Lymphomas) ***	BM	10 - 20 ml	EDTA or heparin	4 °C - RT	RT
		PB	only after consultation (see section 5.1)	EDTA or heparin	4 °C - RT	RT
	Virus diagnostics (qRT-PCR)	BM	3 ml	EDTA	4 °C	RT
		PB	3 ml	EDTA	4 °C	RT
		Urine	at least 1 ml	-	4 °C	RT
		Stool	at least 1 spoonful	-	4 °C	RT
		Swab	swab	dry	4 °C	RT
		Biopsy	at least 1 piece(s)	in 0.9% NaCl solution or PBS	4 °C	RT
		other body fluids	at least 1 ml	-	4 °C	RT
	qPCR Gene rearrangement – ALL, AML	BM	at least 3 ml (5 million cells)	EDTA	4 °C	RT
		PB	at least 3 ml (5 million cells)	EDTA	4 °C	RT

Examination		Suitable material	Required volume	Anticoagulants and special sample collection requirements	Storage until shipment	Shipment
	qPCR Gene rearrangement - CML	BM	at least 3 ml (20 million cells)	EDTA	4 °C	RT
		PB	at least 3 ml (20 million cells)	EDTA	4 °C	RT
	Gene rearrangement pediatric solid tumors	BM	at least 5 ml per puncture site	EDTA	4 °C	4 °C
		Frozen tumor	at least 1 piece(s)	snap-frozen	-80 °C	dry ice
	Chimerism analysis (STR-PCR) * **Typing (before stem cell transplantation)**	PB from recipient and PB from donor	1 ml each	EDTA	4 °C	RT
	Chimerism analysis (STR-PCR) * **Follow-up after stem cell transplantation using whole sample or sorted cells**	BM	2 ml	EDTA	4 °C	RT
		PB	2ml	EDTA	4 °C	RT
SNP array *	**Hematological malignancies**	BM	5 ml	EDTA	RT	RT
		PB	5 ml	EDTA	RT	RT
	T-LBL / Lymphomas	BM	5 ml	EDTA	RT	RT
		Fresh tumor	at least 1 piece(s)	in 0.9% NaCl solution	4 °C - RT	4 °C - RT
		Frozen tumor	at least 1 piece(s)	snap-frozen	-80 °C	dry ice
		Pleural effusion; Ascites	at least 5 million cells	-	RT	RT
		Paraffin block	at least 1 piece(s)	-	RT	RT
		Paraffin curls	5 x 50 µm curls	-	RT	RT
		Paraffin section	4 x 3-5 µm sections	-	RT	RT
	Syndrome diagnostics	PB	2 - 5 ml #	EDTA	RT	RT

Examination		Suitable material	Required volume	Anticoagulants and special sample collection requirements	Storage until shipment	Shipment
	pediatric solid tumors	Fresh tumor	at least 1 piece(s)	in RPMI or 0.9% NaCl solution, shipment within 24 h	4 °C	4 °C
		Frozen tumor	at least 1 piece(s)	snap-frozen	-80 °C	dry ice
		Touch imprints	at least 10 piece(s)	-	RT	RT
		Paraffin block	at least 1 piece(s)	-	RT	RT
		Paraffin curls	5 x 50 µm curls	-	RT	RT
		Paraffin section	4 x 3-5 µm sections	-	RT	RT
		DNA	at least 10 µl (>50 ng/µl)	-	4 °C	4 °C
WES	**Syndrome diagnostics**	PB	2 - 5 ml #	EDTA	RT	RT
Sample preparation for studies	**Please contact the respective study coordinator before shipping samples.**					

*Sub-processes are performed by appropriately qualified contracted laboratories. These are indicated accordingly in the respective report.

For newborns (up to 3 months), 2 ml PB is sufficient.

7. Completing the Request Form

- Patient identification: surname, first name, date of birth, sex (label if applicable)
- Referring institution: name of hospital or institute, responsible physician, approval stamp where applicable (WiGeV)
- Sample material: specify sample type, collection date and, if required, collection site, time and tumor cell content
- Mark or state the reason for referral
- Mark the requested examination
- For study patients: specify the study, study number and time point of sample collection
- Complete any additional required forms where applicable - see website
    - Informed consent: patient identification, signatures of physician and patient with place and date; information on documentation of results, use and storage of residual material, report transmission; preference regarding communication of incidental findings from genome-wide analyses
    - Declaration of cost coverage: details of the payer if examination costs are not covered by the referring institution
- In case of an urgent examination: Mark or indicate this on the request form

8. Storage of Examination Material Prior to Shipment

The time between collection and delivery of fresh material should be as short as possible and should not exceed a maximum transport time of 24 hours!

If the collected material must be temporarily stored after collection, suitable storage conditions must be maintained immediately after collection until transport/shipment, in accordance with the information provided.

9. Packaging, Labeling and Transport

Correct packaging, labeling and transport of the sample material are the responsibility of the referring institution. The applicable domestic / international regulations for the transport of dangerous/biological goods must be considered.

10. Transmission of Medical Reports

Reports are transmitted either on paper or electronically (via the DaMe report portal or by encrypted email). Depending on urgency, results may be transmitted as a preliminary, not yet authorized result or as a final, authorized report.

11. Sample Archiving

Residual primary sample material and/or secondary samples obtained from the former are disposed of after reporting has been completed, at the latest when the legally prescribed retention periods have expired. Upon request, the material can be disposed of earlier, in accordance with legal requirements.