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Primary Sample Collection Manual

Labdia Labordiagnostik GmbH

Versionsnummer: 3

Contents

Abbreviations

Abbreviation	Meaning
µm	Micrometer
0,9% NaCl	Physiological Sodium Chloride
AIPF	Automatic immunofluorescence plus FISH
AML, CML	Acute and chronic myeloid leukaemia
B-ALL, T-ALL	B cell and T cell acute lymphoblastic leukaemia
BM	Bone Marrow
CSF	Cerebro-spinal fluid
DEB	Diepoxybutan
EDTA	Ethylene Diamine Tetra-acetic Acid
EWOG	The European Working Group of MDS and SAA in children (EWOG-MDS/SAA)
FISH	Fluorescence-in-situ-Hybridisation
HE	Haematoxylin-Eosin Stain
HLA	Human leucocyte antigen
mill	million
min.	minimum
ml	milliliter
MNC	Mono-Nuclear Cells
MRD	Minimal Residual Disease
PB	Peripheral Blood
PCR	Polymerase Chain Reaction
qRT-PCR	Quantitative Real Time PCR
RPMI	Roswell Park Memorial Institute Medium
RT	Room Temperature
SCT	Stem Cell Transplant
SNP	Single Nucleotide Polymorphism
STR-PCR	Short tandem repeats - PCR
T-LBL	T cell lymphoblastic leukaemia
WBC	White Blood Cells
WES	Whole Exome Sequencing

1. Contact Information Labdia Labordiagnostik GmbH

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The contact details of staff members from individual departments can be found on our website.

2. General Information on the Organisation

2.1 Opening Hours - Sample Acceptance

Monday – Friday	8:00 – 16:00 Hours
Saturday, Sunday and Bank Holidays - samples will only be accepted from paediatric haematological study patients via St. Anna Children's Hospital	9:00 – 12:00 Hours

In case of urgent samples sent outside of these times the lab needs to be notified in advance.

If samples are delivered outside of the acceptance hours, they will be processed on the following working day. This can limit the analysis of the sample and will be referred to in the report.

2.2 Availability of Clinical Guidance

Employees from the specific departments are available during opening hours for advice on the requirements for each test.

Medical advice on the test request or on the interpretation of the test results by the attending doctor is possible. The information is only given to the referring doctor or to the referring office.

2.3 Policy on the Protection of Personal Information

In order to maintain confidentiality and protect personal information, the data protection regulations applicable in the company are applied. Labdia Labordiagnostik GmbH has officers in the company on the subject of data protection, who regulate and monitor compliance with data protection regulations.

2.4 Feedback – Complaint Management

For feedback of any kind – except for patient-specific information – the Wollen Sie uns etwas mitteilen? - Labdia form on the Labdia website can be used. The feedback is received and assigned to the responsible department and appropriate measures are initiated in the event of a complaint.

3. General Information about this Manual

3.1 Primary Sample Collection Manual

The following information is intended as an aid for correct sample selection and collection. In addition, the optimal storage and transport conditions for the different types of samples are described.

3.2 Pre-analytics

We would like to specifically point out that the quality of our results is significantly influenced by the pre-analytical conditions of the samples. Any shortcomings in the pre-analytical stages cannot be compensated for in further sample processing.

Important pre-analytical steps to consider:

- Suitable sample type and anticoagulant
- Correct sampling procedure (sampling technique) at a suitable sample collection site, including preparation of the patient
- The time the sample is taken
- The volume of the sample
- How the sample will be stored and for how long before transported to us
- Conditions during transportation and duration of transportation

4. Sample Acceptance and Acceptance Criteria

For the best possible sample acceptance and processing, we ask that our sample acceptance criteria be taken into consideration. Clear labelling of all samples taken and paired with the appropriate request form is necessary for error-free identification and is the responsibility of the requesting centre. If a printed patient label is not used, then the sample must be legibly labelled with the surname, first name and date of birth of the patient, as well as the material type / material code (e.g., puncture site, syringe number).

4.1 Acceptance Criteria – Referral Form

- Referring center / provider
- Patient details
- Sample type
- Date and time of sampling
- Referral reason / diagnosis
- Specify the desired investigation / test
- If a declaration of consent is required, it should be filled out accordingly (see the Labdia Labordiagnostik GmbH website)
- According to Section 69 of the Genetic Engineering Act, a genetic analysis may only be carried out if written confirmation is available

4.2 Acceptance Criteria – Sample

- Labelling: Patient data, sample type, date of collection and collection point
- Appropriate sample material for the desired investigation (see point 6)
- Sufficient volume for the desired investigation (see point 6)
- Correct use of anticoagulant (see point 6**Fehler! Verweisquelle konnte nicht gefunden werden.**)
- Suitable transport conditions (see point 6)

If not all acceptance criteria are met, then analysis may be limited. A corresponding note will be included in the report.

Important:

Samples without clear patient identification **cannot** be processed and analysed!

5. Sample Material

Samples are taken exclusively by medically trained staff in accordance with the internal guidelines of the referring centre. Therefore, there is no description of the collection and safe handling including disposal in this sample manual. Study protocols and their requirements for the material may need to be considered.

5.1 Bone Marrow (BM), Peripheral Blood (PB), Ascites, Pleural fluid

5.1.1 Collection Tubes

Depending on the desired investigation, sample material may have to be provided with anticoagulant (See point 6). Please note manufacturer and study guidelines.

Tubes with anticoagulant (ready to use)

- For BM, PB – select anticoagulant according to the requirement for the respective analysis

Tubes without anticoagulant

- BM, PB: Addition of an anticoagulant is necessary: EDTA, Heparin (500 IU/ml), Citrate
- BM biopsy (Punctio sicca): For molecular genetic investigations do not use Formalin. Use 0,9% NaCl solution with Heparin 500 IU/ml
- Ascites and Pleural fluid: Pour into a tube without anticoagulant

PAXgene tubes for Austrian Neuroblastoma patients

- In line with the HR-NBL-1 and HR-NBL-2 studies, 500µl of bone marrow from each puncture site and 500µl of peripheral blood should be added to labelled PAXgene Blood RNA tubes and transported at room temperature.
- PAXgene tubes are available free of charge from the Tumour Biology lab at St. Anna Children's Cancer Research Institute (tumorbiology@ccri.at, +43 1 40470 / 4055).

5.1.2 Venous Blood

- Invert the tube (with anticoagulant) several times immediately after collection.
- Anticoagulant dose – Dependent on investigation and study protocol

5.1.3 Bone Marrow Biopsy and Aspiration

- Invert the tube/syringe (with anticoagulant) several times immediately after collection
- Write the puncture site on the tube

- Label the order the syringes were filled
- Anticoagulant dose – Dependent on investigation and study protocol
- If sufficient bone marrow cannot be obtained in paediatric hemato-oncologic neoplasms (B-ALL; T-ALL; AML), sending peripheral blood is sufficient for flow cytometry, patient-specific qPCR for MRD, FISH, SNP array and for almost all companion research projects if the peripheral blast percentage is >50%. In case of doubt, please contact the study centre and the executing 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.
- In the feathered edge, the cells should be in a monolayer.
- Advantages of this method: Excellent display of single cells → optimal for cytological analysis.
- Disadvantage of this method: Rapid coagulation of the bone marrow → only a limited number of smears can be produced.
- When bone marrow cells are absent, the cellularity and iron content cannot be reliably determined.

5.3 Urine and Faeces

- Urine and faeces samples to be sent in tightly sealed containers.

5.4 Touch Imprints of Tumour Tissue

- Dab the sterile piece of tumour tissue several times onto an adhesive slide with pressure. Do not smear.
- Label the slides with the patient details and date, with a pencil.
- Send the slides in an airtight container at room temperature.
- For FISH analysis, at least 2 slides required. For SNP Array analysis, at least 10 preparations required.

5.5 Tumour tissue – Fresh or Frozen

- Place a piece of sterile tumour in a container with nutrient solution (RPMI) or 0,9% NaCl solution and send on ice.
- Alternatively, the tumour piece can be shock frozen with dry ice and transported on sufficient dry ice.
- Indicate the tumour cell content.

5.6 Paraffinblock, -sections, -rolls

- Paraffin blocks must be labelled with an identifier (e.g., histology number) and can be transported at room temperature. Paraffin blocks will be returned following completion of investigation.
- Alternatively: 3 - 5 Paraffin rolls (20 µm thickness) in a container (1.5 or 2 ml tube) and sent at room temperature.
- For FISH analysis: Minimum 4 slides (per analysis) with 3-5 µm sections, labelled (patient identifier, histology number) with a pencil and sent in an airtight container at room temperature.
- Please always enclose a HE slide with the relevant tumour area marked.
- Indicate the tumour cell content.

6. MATERIAL REQUIREMENTS FOR THE RELEVANT TEST

Investigation / test		Suitable Material	Required Amount	Anticoagulant and Special Requirements for Sampling	Storage Conditions	Transport
Chromosome Analysis	Haematological neoplasms	BM	5 ml	Heparin	RT	RT
		PB	5ml	Heparin	RT	RT
		Tumour, pleural fluid, ascites		fresh (tumour can also be in RPMI or 0,9 % NaCl sol.)	RT	RT
	Congenital Disorders /Birth defects	PB	5 ml #	Heparin	RT	RT
	DEB Test	PB	10 ml	Heparin	RT	RT
Flow Cytometry	Immunological Diagnostics MRD, progression (B-ALL; T-ALL; AML)	BM-Syringe No. 1	min. 1 - 2 ml	Heparin	4 °C - RT	RT
	Immunological Diagnostics EWOG	BM	min. 1 - 2 ml	Heparin	4 °C - RT	RT
	Immunological Diagnostics Immunophenotyping	preferably BM	BM min. 1 - 2 ml	Heparin	4 °C - RT	RT
	Immunological Diagnostics Suspected lymphoma	BM	min. 1 - 2 ml	Heparin	4 °C - RT	RT
		PB	2,5 ml	EDTA	4 °C - RT	RT
		CSF; pleural fluid; ascites: (at the request of the clinic and after consultation)	0,5 – 1 ml CSF, pleural fluid, ascites	No anticoagulant; CSF to be sent within 2 hours	4 °C - RT	RT
	Immunological Diagnostics Presence of blasts in PB	PB	2,5 ml	EDTA	4 °C - RT	RT
	Analysis with Dual Platform Method; Immune status (**Basic analysis**)	BM	2 ml	Heparin	4 °C	RT
		PB	2 ml	EDTA	4 °C	RT
		CSF, pleural fluid, ascites	0,5 – 1 ml CSF, pleural fluid, ascites	No anticoagulant; CSF to be sent within 2 hours	4 °C	RT
	Analysis with Dual Platform Method to demonstrate regeneration after SCT	PB	4 ml	EDTA	4 °C	RT

Investigation / test		Suitable Material	Required Amount	Anticoagulant and Special Requirements for Sampling	Storage Conditions	Transport
	Analysis with Dual Platform Method to detect T-cell subgroups (HLA-DR on CD3 and CD25 on CD4). **Only in combination with basic analysis**	PB	2 ml	EDTA	4 °C	RT
	Analysis with Single Platform Method for quantitative CD34 determination	PB	2 ml	EDTA	4 °C	RT
	Eosin-5'-Maleimid-Binding-Assay (EMA)	PB	2 ml	EDTA; Indicate time of last erythrocyte transfusion **Analysis Tues + Fri**	4 °C	RT
	Leukocyte population sorting for chimerism analysis. Follow-up after SCT in hemato-oncological diseases and immune deficiencies. **Only in combination with basic analysis**	BM	min. 2 ml	Heparin	4 °C	RT
		PB	min. 2 ml	EDTA	4 °C	RT
ELISPOT	Secretion assay to detect virus-specific T cells	PB	4 ml	EDTA or Heparin (Detection only from 20 CD3 / µl; **Sample acceptance Mon-Thurs**	4 °C	RT
Fluorescent In Situ Hybridisation (FISH)	Haematological neoplasms - 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's Lymphoma	BM	2 - 5 ml	EDTA or Heparin	RT	RT
		BM smear	min. 2 slides	fresh	RT	RT
	Lymphoma	BM	2 - 5 ml	EDTA or Heparin	RT	RT
		Touch imprints	min. 4 slides	-	RT	RT
		Pleural fluid; ascites	2 - 5 ml	-	RT	RT
	Chimerism	BM	2 - 5 ml	EDTA or Heparin	RT	RT
		PB	2 - 5 ml	EDTA or Heparin	RT	RT
	Paediatric solid tumours	Tumour fresh	min. 1 piece	in RPMI or 0,9% NaCl solution. Sent within 24 hours	4 °C	4 °C
		Tumour frozen	min. 1 piece	Shock frozen	-80 °C	Dry ice
		Touch imprints	2 slides per FISH Analysis	-	RT	RT
		Paraffin block	min. 1 block	-	RT	RT

Investigation / test		Suitable Material	Required Amount	Anticoagulant and Special Requirements for Sampling	Storage Conditions	Transport
		Paraffin rolls	5 x 50 µm rolls	-	RT	RT
		Paraffin sections	4 x 3 – 5 µm sections	-	RT	RT
	Dysmorphology	PB	2 - 5 ml #	EDTA or Heparin	RT	RT
HPLC	Drug level determination	PB	5 ml	EDTA	4 °C - RT	RT or 4 °C
	Haemoglobinopathy (incl. electrophoresis	PB	4 ml	EDTA	4 °C	RT
Immuncytology (AIPF)	MRD-determination for Neuroblastoma, Rhabdomyosarcoma	BM	min. 5 ml per puncture site	EDTA, in addition 0,5 ml per puncture site in PAXgene Blood RNA (for detailed description see 5.1.1. – point 3), Sent within 24 hours	4 °C	4 °C
		Apheresis	min. 1 ml	Sent within 24 hours	4 °C	4 °C
		other materials (effusion, CSF) following consultation	Effusion 20 ml CSF min. 0,5 ml	Sent within 24 hours	4 °C	4 °C
		Cytospins from BM	2 puncture sites: min. 2 pieces 1 puncture site: 4 pieces	1,0 - 1,2x10^6 MNCs per cytospin	RT	RT
		With sufficient tumour cell infiltration, further genetic analyses (e.g. FISH) can be carried out on this material				
Mutation Screening*	Haematological neoplasms	BM	2 ml	EDTA or Heparin	RT	RT
		PB	2 ml	EDTA	RT	RT
		DNA	after consultation (dependent on query)	-	4 °C	4 °C
		Tumor	min. 1 piece	In 0,9% NaCl solution	4 °C	RT
	Dysmorphology	PB	2 ml	EDTA	RT	RT
		DNA	after consultation (dependent on query)	-	4 °C	4 °C
		Other materials (e.g. oral mucosa, hair, etc.)	Only after consultation	-	Only after consultation	
	T-LBL	BM	5 ml	EDTA or Heparin	RT	RT
		Tumor fresh	min. 1 piece	in 0.9% NaCl solution	4 °C	RT
		Tumor frozen	min. 1 piece	shock frozen	-80 °C	Dry ice
		Pleural fluid; ascites	min 5 mill cells	-	RT	RT
	Paediatric solid tumours *	Tumor fresh	min. 1 piece	in RPMI or 0.9% NaCl solution; send within 24 hours	4 °C	4 °C
		Tumor frozen	min. 1 piece	shock frozen	-80 °C	Dry ice

Investigation / test		Suitable Material	Required Amount	Anticoagulant and Special Requirements for Sampling	Storage Conditions	Transport
		Touch imprints	min. 10 pieces	-	RT	RT
		Paraffin block	1 piece	-	RT	RT
		Paraffin rolls	5x 50 µm rolls	-	RT	RT
		Paraffin sections	4x 3 - 5 µm sections	-	RT	RT
		DNA	min. 10 µl (min. 50 ng/µl)	-	4 °C	4 °C
	BCR::ABL1 TK Kinase domain *	PB	min. 3 ml (20 mill cells)	EDTA	4 °C	RT
PCR	Patient-specific qPCR for MRD determination (B-ALL; T-ALL; AML; Lymphoma) *	BM	10 - 20 ml	EDTA or Heparin	4 °C - RT	RT
		PB	Only following consultation (see point 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	min. 1 ml	-	4 °C	RT
		Stool	min. 1 spoonful	-	4 °C	RT
		Smear	1 slide	dry	4 °C	RT
		Biopsy	min. 1 piece	In 0.9% NaCl solution or PBS	4 °C	RT
		Other materials (fluids)	min. 1 ml	-	4 °C	RT
	qPCR Gene rearrangement – ALL , AML	BM	min. 3 ml (5 mill cells)	EDTA	4 °C	RT
		PB	min. 3 ml (5 mill cells)	EDTA	4 °C	RT
	qPCR Gene rearrangement - CML	BM	min. 3 ml (20 mill cells)	EDTA	4 °C	RT
		PB	min. 3 ml (20 mill cells)	EDTA	4 °C	RT
	Gene rearrangement - Paediatric solid tumours	BM	min. 5 ml per puncture site	EDTA, sent within 24 hours	4 °C	4 °C
		Tumour frozen	min. 1 piece	Shock frozen	-80 °C	Dry ice
	Chimerism Analysis (STR-PCR) * Typing (before SCT)	PB from recipient and PB from donor	1 ml each	EDTA or Heparin	4 °C	RT
	Chimerism Analysis (STR-PCR) *	BM	2 ml	EDTA or Heparin	4 °C	RT
	Follow up after SCT from whole blood/BM or from sorted cells	PB	2ml	EDTA or Heparin	4 °C	RT

SNP Array *	Haematological neoplasms	BM	5 ml	EDTA	RT	RT
		PB	5 ml	EDTA	RT	RT
	T-LBL / Lymphoma	BM	5 ml	EDTA	RT	RT
		Tumour fresh	min. 1 piece	in 0.9% NaCl solution	4 °C - RT	4 °C - RT
		Tumour frozen	min. 1 piece	Shock frozen	-80 °C	Dry ice
		Pleural fluid; ascites	min. 5 mill cells	-	RT	RT
		Paraffin block	min. 1 piece	-	RT	RT
		Paraffin rolls	5x 50 µm rolls	-	RT	RT
		Paraffin sections	4x 3-5 µm sections	-	RT	RT
	Dysmorphology	PB	2 - 5 ml #	EDTA	RT	RT
	Paediatric solid tumours	Tumour fresh	min. 1 piece	in RPMI or 0.9% NaCl solution. Sent within 24 hours	4 °C	4 °C
		Tumour frozen	min. 1 piece	Shock frozen	-80 °C	Dry ice
		Touch imprints	min. 10 pieces	-	RT	RT
		Paraffin block	min. 1 piece	-	RT	RT
		Paraffin rolls	5x 50 µm rolls	-	RT	RT
		Paraffin sections	4 x 3-5 µm sections	-	RT	RT
		DNA	min. 10 µl (>50 ng/µl)	-	4 °C	4 °C
WES *	Dysmorphology	PB	2 - 5 ml #	EDTA	RT	RT
Preparation of materials for studies		We kindly ask you to contact the respective study organisers before sending samples				

* Sub-processes are carried out in specially qualified contract laboratories. These are noted accordingly on the respective findings.

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

7. Filling out the Referral Form

- Patient details: Surname, first name, date of birth, sex. Use a label if possible
- Referring Centre: Hospital/Institute or responsible doctor.
- Sample: type of material, sampling date, and if known - location and time of sampling, tumour cell content
- Mark the reason for the request
- Mark the desired tests
- If necessary, fill out any additional forms that may be required – See Website
    - Letter of consent: Patient name, signatures of patient/guardian and doctor with place and date; Information on the documentation of the results; Use and storage of any remaining material; Transfer of results; Preference for being informed of incidental findings through genome-wide analysis
    - Declaration of payer of cost: Information on the payer of the tests if they are not to be covered by the referring centre
- In the case of an urgent investigation: Mark it on the form or add a note

8. Storage of Samples before Transport

The interval between sampling and sending of the fresh material should be as short as possible, and the transportation duration should not exceed 24 hours!
If the sample material obtained must be stored temporarily after the sample has been taken, care must be taken to ensure that suitable storage conditions are realised immediately after taking it until it is transported, in accordance with the information provided.

9. Packaging, Labelling and Transport

The correct packaging, labelling and transport of the sample material is the responsibility of the referring centre. The national / international regulations for the transport of dangerous/biological goods must be considered.

10. Reports

The report is sent in written (paper-based) or electronic form, either as a provisional, non-verified analysis result (in the case of high urgency) or as a verified finding.
When specifying a fax number for reports to be sent to, it must be ensured that your fax machine is set up in a protected location.

11. Sample Archive

Remaining primary material and/or secondary samples obtained shall be disposed of at the earliest after completion of the diagnosis, but at the latest after the statutory deadlines have expired. If desired, disposal is possible beforehand due to legal regulations.