

EARCO BIOBANKING STANDARD OPERATING PROCEDURES (SOPs)

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SOP FOR PLASMA BIOBANKING

Blood Collection

Collection time: preferably in the morning. Fasting is not required. Record the time of collection.

Venipuncture site: preferably antecubital vein.

Type of tubes: plasma tubes with EDTA.

Processing of the blood sample between collection and storage

Tube handling: gently invert the collection tubes 5-10 times at room temperature immediately after collection to ensure proper mixing.

Time between collection and centrifugation: centrifugation should ideally be performed within 1 hour of collection - preferably immediately after gentle inversion. In all cases, centrifugation must occur within a maximum of 4 hours post-collection.

Pre-centrifugation storage conditions: samples should be maintained at room temperature until centrifugation. If the delay between collection and processing exceeds 4 hours, samples must be stored at 4°C.

Centrifugation parameters: centrifuge samples at room temperature, at 2000 x g for 10 minutes.

Post-centrifugation handling: aliquoting and storage must be performed immediately following centrifugation.

Storage

Tube material: use polypropylene tubes for sample storage.

Tube and aliquot volumes: preferred tube volumes are 0.5 mL (500 µL). Acceptable volume range is 0,1 mL (100 µL) (minimum) to 2 mL (maximum). Tubes should ideally be filled to approximately 75% capacity to minimize the risk of freeze-drying.

Labeling: Label aliquots using waterproof and freeze-resistant labels whenever possible.

Storage temperature: store aliquots at -80°C for long term preservation.

Preservation

Each plasma aliquot must be thawed only once; refreezing after thawing should be avoided.

Quality control

Use sterile single-use material and wear appropriate personal protective equipment (e.g. lab coat, disposable gloves) during sample handling and processing.

All equipment used for sample collection and processing (e.g., centrifuges, pipettes) must undergo regular calibration and maintenance in accordance with manufacturer guidelines.

Upon sample receipt, verify the following:

- Aliquots have consistent fill volumes.
- Samples have properly mixed with any pre-added reagents.
- No visible signs of haemolysis are present (perform visual inspection).

Refrigerators and freezers used for sample storage must be equipped with a continuous temperature monitoring system. If such a system is not in place, technical personnel must perform and document daily temperature checks.

Storage units must be located in air-conditioned rooms maintaining a stable ambient temperature, ideally below 20°C, to prevent over-heating and ensure equipment performance.

SOP FOR SERUM BIOBANKING

Blood collection

Collection time: preferably in the morning. Fasting is not required. Record the time of collection.

Venipuncture site: preferably antecubital vein.

Type of tubes: serum tubes (no anticoagulants). Record whether you use tubes with or without gel separator.

Processing of the blood sample between collection and storage

Coagulation step: blood collection tubes must be kept in an upright position at room temperature for 30-60 minutes to allow complete coagulation.

Time between collection and centrifugation: a minimum of 30 minutes is required post-collection to allow clot formation. The optimal time window for centrifugation is 30-60 minutes after collection. In all cases, centrifugation must occur within a maximum of 4 hours post-collection.

Pre-centrifugation storage conditions: sample should be maintained at room temperature until centrifugation. If the delay between collection and processing exceeds 4 hours, samples must be stored at 4°C.

Centrifugation parameters: centrifuge samples at room temperature, at 2000 x g, for 10 minutes.

Post-centrifugation handling: aliquoting and storage must be performed immediately following centrifugation.

Storage

Tube material: use polypropylene tubes for sample storage

Tube and aliquot volumes: preferred tube volumes are 0.5 mL (500 µL). Acceptable volume range is 0,1 mL (100 µL) (minimum) to 2 mL (maximum). Tubes should ideally be filled to approximately 75% capacity to minimize the risk of freeze-drying.

Labeling: Label aliquots using waterproof and freeze-resistant labels whenever possible.

Storage temperature: store aliquots at -80°C for long term preservation.

Preservation

Each plasma aliquot must be thawed only once; refreezing after thawing should be avoided.

Quality control

Use sterile single-use material and wear appropriate personal protective equipment (e.g. lab coat, disposable gloves) during sample handling and processing.

All equipment used for sample collection and processing (e.g., centrifuges, pipettes) must undergo regular calibration and maintenance in accordance with manufacturer guidelines

Upon sample receipt, verify the following:

- Aliquots have consistent fill volumes.
- Samples have properly mixed with any pre-added reagents.
- No visible signs of haemolysis are present (perform visual inspection).

Refrigerators and freezers used for sample storage must be equipped with a continuous temperature monitoring system. If such a system is not in place, technical personnel must perform and document daily temperature checks.

Storage units must be located in air-conditioned rooms maintaining a stable ambient temperature, ideally below 20°C, to prevent over-heating and ensure equipment performance.

SOP FOR BRONCHOALVEOLAR LAVAGE (BAL)

BAL collection

Patient fasting is not required. Bronchoalveolar lavage (BAL) fluids must be collected in sterile containers equipped with a secure lid or stopper.

The collected biological material should be transported to the laboratory as soon as possible, and within 30 minutes of collection.

Transport the container in an upright position, at room temperature.

If processing is delayed beyond 30 minutes, store at 4°C until further handling.

BAL processing

Pre-processing steps: record the volume of physiological saline (NaCl) instilled and recovered before beginning processing. All steps must be performed under a laminar flow hood.

Filtration: Filter BAL fluid through a sterile gauze or a suitable filter to remove aggregated debris.

Record the volume of the filtered BAL.

First centrifugation: centrifuge the filtered fluid at 300 x g for 10 minutes at room temperature. Collect and store the supernatant (bronchoalveolar lavage-fluid, BAL-f) in sterile tubes at -20°C.

Cell pellet handling: re-suspend the cell pellet in 50 mL of PBS or Physiological Saline (NaCl).

Centrifuge again the sample at 300 x g for 10 minutes at room temperature. Discard the supernatant and re-suspend the resulting cell pellet in an appropriate volume of PBS or NaCl to achieve the desired concentration.

Cell counting and slide preparation: perform a total cell count. For each slide, prepare $0,6 \times 10^6$ cells in a final volume of 50 μ l (diluted in PBS or NaCl). Use a Cytospin centrifuge for 4 minutes at 1800 x g to deposit the cells on slides.

Slide fixation: Depending on downstream applications (e.g. staining), slides may be fixed using a Cytological fixative spray or left unfixed.

Storage conditions

BAL-f samples: store BAL-f aliquots in suitable tubes at -80°C, for long term preservation.

Unstained slides: stored unstained slide in the appropriate slide holders at room temperature for long term storage.

Preservation

Each BAL-f aliquot must be thawed only once; refreezing after thawing is not permitted.

Stained slides cannot be re-stained and must be prepared correctly on the first attempt.

Quality control

To obtain a slide of sufficient quality for analysis, each slide should contain $0,6 \times 10^6$ cells.

Ensure accurate cell counting and proper slide preparation techniques to guarantee readability and diagnostic reliability.

Use sterile single-use material and wear appropriate personal protective equipment (e.g. lab coat, disposable gloves) during sample handling and processing.

All equipment used for sample collection and processing (e.g., centrifuges, pipettes) must undergo regular calibration and maintenance in accordance with manufacturer guidelines.

Upon sample receipt, verify the following:

- Aliquots have consistent fill volumes.

Refrigerators and freezers used for sample storage must be equipped with a continuous temperature monitoring system. If such a system is not in place, technical personnel must perform and document daily temperature checks.

Storage units must be located in air-conditioned rooms maintaining a stable ambient temperature, ideally below 20°C, to prevent over-heating and ensure equipment performance.

SOP FOR PERIPHERAL WHOLE BLOOD BIOBANKING FOR DNA EXTRACTION

Blood Collection

No special preparation of the patient is required. Fasting is not required.

Collection time: preferably in the morning. Record date of collection.

Venipuncture site: preferably antecubital vein.

Type of tubes: blood/plasma tubes with EDTA.

- Mix carefully immediately after collection by rotating the tube 8-10 times upside down.

Processing of the blood sample between collection and storage

Transport blood samples to the laboratory in a closed protective container, maintaining them in an upright position at room temperature (18 - 25°C), preferably within 15-30 minutes of collection.

If immediate processing is not possible, store samples at 2 - 8°C (refrigerator temperature) for up to 5 days prior to DNA extraction or store at -20°C for long term storage before DNA extraction.

Sample handling temperature: room temperature.

Before processing, gently invert the blood collection tubes 5-10 times to ensure the proper mixing.

Aliquot the blood samples into transparent polypropylene tubes suitable for long-term storage. Freeze aliquots within 1h after aliquoting.

Optional procedures:

- perform DNA extraction from one aliquot, according to the validated laboratory protocol or manufacturer's recommendations, depending on the commercial DNA extraction kit used.
- prepare Dried Blood Spot (DBS) samples for storage (refer to the SOP for DBS preparation).

Storage conditions

Tube material: transparent polypropylene tubes.

Tube and aliquot volumes: various, from 0.25 mL (250 µL) up to 10 mL.

Aliquot Volume: minimum 200 µL, maximum 10 mL.

- Preferably, adjust the aliquot volume according to the input volume required for the DNA extraction method used in the laboratory.
- Tubes should ideally be filled to approximately 75% capacity to minimize the risk of freeze-drying.

Labeling: Label aliquots using waterproof and freeze-resistant labels whenever possible.

Storage temperature: store aliquots at -20°C for up to 6 weeks or at -80°C for long term preservation.

Preservation

To prevent cell damage upon deep-freezing it is recommended to initially freeze the blood tubes at -20°C for a minimum of 24 hours and then transfer the tubes to a -80°C freezer.

Each whole blood aliquot must be thawed only once; refreezing after thawing should be avoided.

Quality control

Use sterile single-use material and wear appropriate personal protective equipment (e.g. lab coat, disposable gloves) during sample handling and processing.

All equipment used for sample collection and processing (e.g., centrifuges, pipettes) must undergo regular calibration and maintenance in accordance with manufacturer guidelines.

Upon blood sample receipt, verify the following:

- Aliquots have consistent fill volumes.
- Samples have properly mixed with any pre-added reagents.
- No visible signs of clotting or haemolysis are present (perform visual inspection).

Refrigerators and freezers used for sample storage must be equipped with a continuous temperature monitoring system. If such a system is not in place, technical personnel must perform and document daily temperature checks.

Storage units must be located in air-conditioned rooms maintaining a stable ambient temperature, ideally below 20°C, to prevent over-heating and ensure equipment performance.

SOP FOR DNA BIOBANKING

Biological material for DNA extraction

- peripheral whole blood collected in EDTA tube (refer to the SOP for peripheral whole blood biobanking for DNA extraction)
- dried blood spot (DBS) samples
- buccal swabs

Sample processing

DNA extraction should be performed in accordance with the protocol validated in the laboratory or following the manufacturer's instructions, depending on commercial kit used.

For high-quality nucleic acid extraction, whether automated or manual, it is recommended to use commercially available kits based on magnetic bead separation or column-based ion exchange methods. Phenol-chloroform extraction is not recommended, particularly for high-throughput PCR-based molecular techniques such as next-generation sequencing.

Aliquot the extracted DNA into polypropylene tubes suitable for long-term storage.

Whenever possible, label aliquots with waterproof and freeze-resistant labels.

Storage conditions

Tube type: transparent polypropylene tubes, preferably low-binding

Tube volume: preferably 1,5 mL (1500 μ L)

Aliquot volume: 10 μ L to 50 μ L

Storage temperature:

- 2 - 8°C: for storage up to 6 months
- from $\leq -20^{\circ}\text{C}$ to $\leq -80^{\circ}\text{C}$ for long-term storage, indefinitely

Preservation

To maintain DNA integrity, minimize freeze-thaw cycles.

Quality control

Use sterile single-use material and wear appropriate personal protective equipment (e.g. lab coat, disposable gloves) during sample handling and processing.

All equipment used for sample collection and processing (e.g., centrifuges, pipettes) must undergo regular calibration and maintenance in accordance with manufacturer guidelines.

Refrigerators and freezers used for sample storage must be equipped with a continuous temperature monitoring system. If such a system is not in place, technical personnel must perform and document daily temperature checks.

Storage units must be located in air-conditioned rooms maintaining a stable ambient temperature, ideally below 20°C, to prevent over-heating and ensure equipment performance.

Assessment of DNA concentration and purity

1) Initial assessment – spectrophotometric analysis.

DNA purity should be first evaluated using absorbance ratios measured via spectrophotometry.

- A₂₆₀/A₂₈₀ ratio: optimal value ~1.8; acceptable if >1.7.
A ratio <1.7 suggests the presence of residual protein, acidic phenol, or other contaminants associated with the extraction protocol.
A ratio >2.0 indicates RNA contamination or alkaline/basic contamination.
- A₂₆₀/A₂₃₀ ratio; optimal range 1.8–2.2, acceptable if >1.5
A ratio <1.5 indicates contamination by organic compounds or salts, such as guanidine isothiocyanate, carbohydrates, phenol, EDTA, or lipids.

Note: spectrophotometry-based quantification tends to overestimate DNA concentration due to its inability to distinguish between DNA and other nucleic acids or contaminants.

2) Accurate Quantification – Fluorometric Assay

To obtain a more accurate measurement of DNA concentration, perform quantification using fluorimeter assay (e.g. Qubit/Quantus fluorimeter), following the manufacturer's instructions. Fluorimeter assays are highly specific and sensitive for double-stranded DNA (dsDNA) as they utilize fluorescent dyes that bind specifically to these molecules. These assays typically provide **lower and more accurate values** compared to spectrophotometry.

3) DNA integrity assessment (optional)

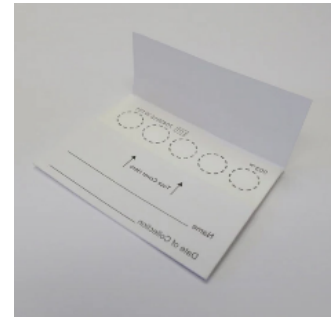
To evaluate the integrity of nucleic acids, use microfluidic electrophoretic separation (e.g. on Agilent Bioanalyzer), according to the manufacturer's instructions or real-time PCR (qPCR) assays.

SOP FOR DRIED BLOOD SPOTS (DBS) BIOBANKING

Biological material used for DBS

- Peripheral whole blood collected in EDTA tube is the preferred biological material for DBS preparation (refer to the SOP for peripheral whole blood biobanking for DNA extraction for detailed procedures); preferred venipuncture site: antecubital vein; patient fasting is not required.
- Capillary blood obtained via fingertip puncture may also be used, although this method is less recommended due to potential variability in sample quality and volume.

Collection consumables - collection card (filter paper) suitable for blood spot collection, in example:



Application of blood samples to filter paper for DBS preparation

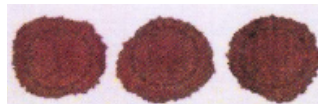
Blood should be spotted onto the filter paper as soon as possible after collection.

The collection card must be clearly labelled e.g. with the patient's identification code, and the date of sample collection.

To prevent contamination of the filter paper, gloves must be worn when handling it, and the sample application area must not be touched.

- For venous blood, gently mix the tube by inverting 5-10 times before application. Using an automatic pipette with a disposable filtered tip, apply 50 µl of blood to the centre of each pre-printed circle on the filter paper; do not smear or rub the blood onto the paper;

example layout:



- For capillary blood, apply directly from the puncture site onto the card.

Drying procedure: place the collection card on a horizontal, exposed, non-absorbent surface, away from direct sunlight. Do not use heat (e.g., radiators or dryers) to accelerate the drying process. Allow the blood spots to air-dry for a minimum of 4 hours at room temperature.

To prevent cross-contamination do not stack collection cards on top of each other during the drying process.

Transportation of DBS samples for laboratory testing

Transport should be carried out at room temperature. The DBS card must be secured in a plastic or aluminum pouch and stored within 7 days of sample collection.

DBS storage conditions

Dried Blood Spot (DBS) cards should be stored at room temperature (18 - 25°C), in a dry, well-ventilated area, away from heat sources (e.g. radiators) and direct sunlight.

For biochemical analyses: DBS samples may be stored for up to 1 month at room temperature.

For DNA-based analyses: DBS samples can be stored long-term, with demonstrated stability for at least 20 years under appropriate conditions.

Quality control

Use sterile single-use material and wear appropriate personal protective equipment (e.g. lab coat, disposable gloves) during sample handling and processing.

Only dedicated filter paper specifically designed for biological sample collection must be used.

Collection cards are intended for single use only. Reusing cards is strictly prohibited. Each card must be used for only one patient.

SOP FOR FRESH TISSUE BIOBANKING

Material collection

Material collection at time of scheduled clinical procedure; communicate with responsible personnel to advise when tissue will be collected and to arrange for timely processing. Patient fasting is not required.

Tissue resection/biopsy samples should be taken according to the doctor's instructions and not preserved. The entire process of surgical sample collection must be carried out under sterile conditions to avoid contamination of the collected material.

Transportation to laboratory for processing in a closed sterile container with phosphate buffered saline, placed in a protective box that maintains an internal temperature of 0-4°C (e.g. on ice), immediately after resection (should be processed within 30 minutes of collection).

- Caution: For establishing immediate cell culture, tissue sample should be delivered immediately after collection, otherwise, stored for up to 24 hours at room temperature in appropriate medium/solution.

Processing

Fresh tissue should be processed and frozen as soon as possible after surgical removal to maintain cell viability and prevent degradation; optimally tissue should be snap-frozen within 30 minutes from resection. Do not freeze the tissue directly on ice.

Label the sterile cryopreservation tubes with waterproof, freeze-resistant and liquid nitrogen-resistant labels.

Use clean forceps to remove tissue specimen from collection tube. Rinse in new tube with phosphate buffered saline. Place specimen on clean cutting surface dish and cut it into small pieces (e.g. tissue cubes sized 0.5 cm³, thin slices of 2-3 mm). If the sample is too large in size, longer freezing time will result in ruined morphology.

- Place each tissue specimen in an empty dry cryotube/vial, without additives.
- Immediately submerge the cryotube/vial into liquid nitrogen until deep-frozen. The specimen should freeze within 30-60 seconds.
- Once snap frozen, transfer the samples for storage (on dry ice if not close by) to the designated -80°C freezer.

OR

- Suspend small and thin tissue fragments (e.g. 2-3 mm) in cryopreservation solution composed of 10 % DMSO and 90 % Fetal Bovine Serum (FBS) and aliquot them into cryotubes/vials.
- Transfer the cryotubes/vials to storage boxes and store initially at -20°C for 2 h, followed by at -80°C for 22 h before ultimately transferring them to liquid nitrogen for long-term storage.

Snap frozen tissue is suitable for preparation of DNA, RNA and protein.

- Optionally: Homogenize the tissue and perform nucleic acid extraction according to the protocol validated in the laboratory or manufacturer's recommendations, depending on commercial kit used.

Storage conditions

Type of tubes to be used: sterile cryopreservation tubes/vials, plastics appropriate for deep-freezing conditions; tube volume depending on tissue size.

Volume of tubes: various, from 0.5 mL (5000 µL) up 5 mL.

Storage temperature:

- $\leq -20^{\circ}\text{C}$ for short-time storage, up to 6 weeks
- $\leq -70^{\circ}\text{C}$ for long-time storage, indefinitely
- cryopreservation using liquid nitrogen, for long-time storage, indefinitely

Preservation

Avoid repeated freeze-thaw cycles of the material.

Quality control

Use sterile single-use material; wear personal protective equipment (lab coat, single use gloves).

Ensure that the resected tissue never desiccates or is contaminated by surrounding tissue or other samples. Use clean scalpel / scissors and forceps between samples to avoid cross contamination between samples.

The refrigerators and freezers in the storage unit should be equipped with a temperature monitoring system, otherwise the technical staff should run daily checks. They should be kept in an air-conditioned space maintaining a constant ambient temperature, optimally $< 20^{\circ}\text{C}$, to avoid overheating.

SOP FOR LIVING CELLS BIOBANKING

Material collection

The entire process of sample collection and processing must be carried out under sterile conditions to avoid contamination of the collected material.

It is recommended that the collected cell sample be cultured first (according to the laboratory's recommendations and procedures) to increase its quantity before biobanking. Adherent cells need to be detached from the culture plates, harvested and resuspended in the complete growth medium for further processing.

Processing of living cells:

The general freezing method is the same for adherent and suspension cells, except that adherent cells need to be removed from the culture plates before starting the freezing procedure.

Freeze cells in log phase, at a high concentration of at least 90% viability, and at as low a passage number as possible.

All processing must be carried out under a laminar flow hood.

- Prepare freezing medium and store at 2 - 8°C until use.
- Transfer the cell suspension to a sterile centrifuge tube of appropriate size and centrifuge at room temperature for 5 to 10 minutes at approximately 100–400 rcf (centrifugation speed and duration varies depending on the cell type).
- Remove the supernatant without disturbing the cell pellet.
- Resuspend the cell pellet in cold freezing medium at the recommended viable cell density for the specific cell type.
- Dispense aliquots of the cell suspension into labeled sterile cryogenic storage vials.
- Optimally, cryovials should be placed in a rate controlled freezing container allowing for slow freezing by reducing the temperature at approximately 1°C per minute until reaching -80°C overnight.

Alternatively, place the cryovials containing the cells in a container filled with isopropanol (an isopropanol chamber) and store them at -80°C overnight.

- The next day, transfer the cryovials with frozen cells into a liquid nitrogen tank for long-term storage; store them in the gas phase above the liquid nitrogen below -130°C .
Alternatively, if liquid nitrogen storage is impossible, cryovials may be kept frozen at -80°C for short-term storage before storing in liquid nitrogen.
- Optionally: perform nucleic acid extraction from the cell pellet collected in a sterile polypropylene tube, according to the protocol validated in the laboratory or manufacturer's recommendations, depending on commercial kit used.

Storage conditions

Type of tubes to be used: sterile cryopreservation tubes/vials, plastics appropriate for deep-freezing conditions.

Volume of tubes: various, from 1 ml (1000 μL) up 5 mL.

Optimal aliquot storage volume: 1 ml (1000 μL).

Caution: Report cell numbers for each tube.

Store the cell sediment in cryopreservation solution.

Storage temperature:

- optimally, $< -130^{\circ}\text{C}$ cryopreservation in liquid nitrogen, stored indefinitely
- $\leq -70^{\circ}\text{C}$ for short-time storage, if liquid nitrogen storage is impossible

Preservation

Avoid repeated freeze-thaw cycles of the material.

Optimal freezing conditions depend on the cell line in use; for detailed protocols, always refer to the cell-specific product insert.

Cryoprotective agents reduce the freezing point of the medium and slow the cooling rate, greatly reducing the risk of ice crystal formation, which can damage cells and cause cell death. Media for cryopreservation typically include a base medium, a cryopreservative, and a protein source. The cryopreservative and protein protect the cells from the stress of the freeze-thaw process.

Care must be taken to minimise the overall processing time (ideally < 4 hours) from the point of harvest to the point of freezing. Otherwise, the yield of functional cells post-thaw may be diminished. Prolonged exposure (> 30 minutes) to hypothermic temperatures ($< 35^{\circ}\text{C}$) will result in a cold shock

response in mammalian cells. Adherent cells also experience stress when maintained without a surface to attach to and proliferate upon.

Quality control

Use sterile single-use material and wear appropriate personal protective equipment (e.g. lab coat, disposable gloves) during sample handling and processing.

Ensure that the resected tissue is never allowed to dry out and is protected from contamination by surrounding tissue or other biological samples. To prevent cross contamination, always use clean and sterile instruments (e.g. scalpel, scissors, forceps) for each individual samples.

Refrigerators and freezers used for sample storage must be equipped with a continuous temperature monitoring system. If such a system is not in place, technical personnel must perform and document daily temperature checks.

Storage units must be located in air-conditioned rooms maintaining a stable ambient temperature, ideally below 20°C, to prevent over-heating and ensure equipment performance.

SOP FOR FORMALIN-FIXED PARAFFIN-EMBEDDED (FFPE) TISSUE FRAGMENTS
OR CELLS (CYTOBLOCKS) BIOBANKING

Material collection

Material collection at time of scheduled clinical procedure; communicate with responsible personnel to advise when tissue will be collected and to arrange for timely processing. Patient fasting is not required.

Tissue resection/biopsy samples should be taken according to the doctor's instructions and not preserved. The entire process of surgical sample collection must be carried out under sterile conditions to avoid contamination of the collected material.

Transportation to laboratory for processing in closed container filled with an appropriate volume of fixative (10% buffered formalin solution with a neutral pH), using a ratio of 10 times greater the volume of fluid relative to the size of the organ/tissue. The container with the material contained in appropriate volume of fixative must be tightly closed and transported to the pathology laboratory at room temperature on the day of collection, as soon as possible.

- Containers should meet the requirements of an in vitro diagnostic medical device, adapted to the size of the collected material, enabling easy immersion of the material in the appropriate amount of formalin.
- The container with the material contained in it must be tightly closed and appropriately labelled.
- The delivered material should be stored at room temperature (no higher than 25°C) before processing.

Processing

Fixation of tissue should be undertaken as soon as possible, optimally, within 4 hours from resection.

The fixative used for tissue material is 10% neutral pH (7.2–7.4) phosphate buffered formalin solution. It is important that the fixative is buffered to avoid the formation of formaldehyde pigment on blood rich tissues.

The volume of the fixative should be at least 10 times greater than the volume of the tissue (i.e. > 10 ml for every gram of tissue). If needed, dissect the tissue to optimum size before fixation to ensure adequate penetration of the fixative.

Perform fixation at room temperature ($\leq 25^{\circ}\text{C}$).

The fixation time is counted from the moment the material is placed in formalin until the tissue samples are taken for further technical processing in the pathology lab. The rate of tissue fixation in a formalin solution is approximately 1 mm per hour. Excessively long storage in fixative will lead to tissue autolysis.

- Large biopsy material should be processed in the pathology lab within 24 to 72 hours.
- Small biopsy material and cytoblocks should be processed within 6 to 48 hours.
- Large specimens may be processed before 24 hours have passed, but not earlier than 12 hours, provided that they have been properly pretreated to allow effective fixation.

Formalin fixation and paraffin embedding (FFPE) should be performed in accordance with the protocol validated in the laboratory. The protocol should include following steps:

- Cut the tissue into small pieces to ensure adequate penetration of the fixative, and place them in labeled cassettes with secured lids.
- Place the cassettes in 10% neutral pH buffered formalin solution. Record time tissue spent in fixative.
- After formalin fixation, perform tissue dehydration through ascending alcohol (ethanol) series, clearing by treatment with xylene, and wax impregnation.
- Perform definite paraffin embedding for long-term storage of paraffin blocks with fixed tissue.

For further processing, use a microtome to cut tissue into thin sections between 5 - 10 μm and place them in labeled 1.5 mL capped tubes.

- You can store the tubes with FFPE long-term or perform nucleic acid extraction from FFPE sections accordingly to the procedure validated in the laboratory or according to the manufacturer's recommendations, depending on commercial kit used.

Storage conditions

Store paraffin blocks at or below room temperature. Prevent exposure to sun or extreme temperature variance. Store blocks in moisture resistant cardboard boxes or plastic storage boxes.

Storage temperature:

Paraffin blocks – at room temperature ($\leq 25^{\circ}\text{C}$), indefinitely

FFPE/cytoblock sections in 1,5 mL tubes at:

- 2 - 8 $^{\circ}\text{C}$ up to 3 days
- $\leq -70^{\circ}\text{C}$ for long-time storage

Preservation

Do not use tissue specimens that are over 8 mm in thickness for fixing procedure, as they may not be adequately fixed.

Use low melt paraffin for embedding, as it will improve the quality of nucleic acids.

Paraffin blocks storage in temperatures $>25^{\circ}\text{C}$ may cause nucleic acid degradation within preserved cells.

Storing FFPE blocks at $\leq -70^{\circ}\text{C}$ is highly recommended for long-term storage to preserve the quality of DNA and RNA for molecular analysis, as storage at lower temperatures slows down the degradation of nucleic acids.

Quality control

Use sterile single-use material and wear appropriate personal protective equipment (e.g. lab coat, disposable gloves) during sample handling and processing.

Ensure that the resected tissue is never allowed to dry out and is protected from contamination by surrounding tissue or other biological samples. To prevent cross contamination, always use clean and sterile instruments (e.g. scalpel, scissors, forceps) for each individual samples.

Refrigerators and freezers used for sample storage must be equipped with a continuous temperature monitoring system. If such a system is not in place, technical personnel must perform and document daily temperature checks.

Storage units must be located in air-conditioned rooms maintaining a stable ambient temperature, ideally below 20°C , to prevent over-heating and ensure equipment performance.