

Standard Operating Procedure (SOP)

Processing of Plasma Samples for the BioBladder Study

1. Purpose

This SOP describes standardized procedures for the collection, handling, processing, and storage of plasma samples, including ctDNA/germline DNA samples and other plasma samples, within the BioBladder study.

2. Scope

This procedure applies to all personnel involved in sample collection and laboratory processing related to the BioBladder study.

3. Responsibilities

- Study personnel are responsible for correct collection, labeling, and timely transport of samples to the laboratory.
- Laboratory staff are responsible for processing samples according to the centrifugation, separation, aliquoting, and storage procedures described in this SOP.

4. Materials and Equipment

- 4 × 10 mL EDTA tubes for each sampling occasion
- Centrifuge capable of operating at 1500 g and 2400 g
- Falcon tubes
- Labeling materials including Study name, Patient ID/Code, Date
- Pipettes and sterile pipette tips (for aliquoting procedures)
- -80 °C freezer

5. Safety Considerations

- Use appropriate PPE: lab coat, gloves, and eye protection.
- Handle all human specimens as potentially infectious.
- Dispose of biological waste according to institutional guidelines.

6. Procedure

A. Plasma Samples for ctDNA / Germline DNA

6.1 Sample Collection

1. Collect 2 × 10 mL EDTA blood tubes.
2. Immediately invert each tube 8–10 times.
3. Allow tubes to stand no longer than 30 minutes at room temperature before centrifugation.

6.2 Centrifugation

1. First centrifugation: 1500 g for 10 minutes.
2. Transfer plasma to Falcon tubes without disturbing the buffy coat.
3. Second centrifugation: 2400 g for 15 minutes.

6.3 Separation and Storage

- Separate plasma and buffy coat into designated tubes.
- No aliquoting is performed for these samples.
- Immediately freeze both plasma and buffy coat at $-80\text{ }^{\circ}\text{C}$.

6.4 Labeling

Each tube must be labeled with:

- Study name (BioBladder)
- Patient ID/Code and time point (T0, T1, S0, etc.)
- Description of sample (ctDNA or buffy coat)
- Date of collection

B. Other Plasma Samples

6.5 Sample Collection

1. Collect $2 \times 10\text{ mL}$ EDTA blood tubes.
2. Invert tubes 8–10 times immediately after collection.
3. Allow tubes to stand no longer than 30 minutes before processing.

6.6 Centrifugation

1. First centrifugation: 1500 g for 10 minutes.
2. Transfer plasma to Falcon tubes.
3. Second centrifugation: 2400 g for 15 minutes.

6.7 Aliquoting and Storage

1. Aliquot plasma into $700\text{ }\mu\text{L}$ portions.
2. Freeze aliquots at $-80\text{ }^{\circ}\text{C}$ immediately.
3. Avoid repeated freeze–thaw cycles.

6.8 Labeling

Each aliquot must be labeled with:

- Study name (BioBladder)
- Patient ID/Code and time point
- Description of sample (plasma or buffy coat)
- Date of collection

7. Storage Requirements

- Store all samples in a $-80\text{ }^{\circ}\text{C}$ freezer.
- Minimize door openings to maintain stable temperature.
- Maintain storage records according to lab standards.

8. Documentation

- Record all sampling events, centrifugation times, aliquot numbers, and storage locations.

- Document any deviations and report to study coordinator.

Standard Operating Procedure (SOP)

Handling of Fresh Tumor Tissue for the BioBladder Study

1. Purpose

This SOP describes the procedures for the collection, handling, and processing of fresh tumor tissue for the BioBladder study.

2. Scope

This SOP applies to pathologists, fresh tissue room personnel, research nurses, and staff involved in handling BioBladder tumor tissue samples.

3. Responsibilities

- Pathologists identify and extract tumor tissue.
- Fresh tissue room staff provide sterile instruments and distribute tissue into study tubes.
- Research nurses manage prioritization, storage, and transport to -80°C .
- All personnel follow documentation and labeling guidelines.

4. Materials and Equipment

- Sterile forceps and scalpels
- Labeled study tubes (Priority 1–3)
- RNAlater tube (Priority 1)
- Falcon 15 mL tubes
- Liquid nitrogen container (Priority 2)
- Refrigerator (4°C)
- -80°C freezer
- Biobank documentation sheets
- LabWare system

5. Procedure

5.1 Pathology Handling

1. After cystectomy, the specimen is delivered to pathology.
2. Identify suspected tumor tissue and collect a representative sample.
3. If tumor >1 cm:
 - Collect tissue for diagnostics and BioBladder research.
 - Provide the largest possible intact piece (>1 cm) for research.
4. If tumor <1 cm:
 - Prioritize diagnostics.
 - Provide research tissue only if sufficient material remains.

5.2 Fresh Tissue Room Handling

1. Samples will be labeled "BioBladder Study."

2. Notify the research nurse immediately at 08–123 74 397.
3. Provide sterile forceps and scalpel to the physician.
4. Physician cuts tumor into ≥ 3 pieces ($\sim 2 \times 2 \times 2$ mm), min 2 mm in one dimension.
5. Distribute pieces evenly into tubes 1–3.
6. If insufficient tissue, prioritize Tube 1 > Tube 2 > Tube 3.
7. Document extraction in Biobank binder and register in LabWare.

5.3 Research Nurse Handling

Priority 1:

- Store Tube 1 (RNAlater) in refrigerator.
- Between Day 1–3, transfer tissue to a 15 mL Falcon tube and freeze at -80 °C.

Priority 2:

- Snap-freeze and store at -80 °C.

Priority 3:

- Store at 4 °C until pickup; long-term storage at -80 °C.

6. Labeling Requirements

Each tube must contain:

- Study name (BioBladder)
- Patient ID/Code and sampling type
- Date of sampling

7. Storage

- Priority 1: -80 °C after incubation in RNAlater
- Priority 2: Snap-frozen, stored at -80 °C
- Priority 3: Refrigerated until pickup, then -80 °C

8. Documentation

- All samples logged in Biobank binder and LabWare.
- Deviations must be documented and reported.

9. Contact Information

Dr. David Krantz – david.krantz@regionstockholm.se

Research Nurse Katrin Fines-Hamrin – katrin.fines-hamrin@regionstockholm.se

Prof. Anders Ullén – anders.ullen@regionstockholm.se

Dr. Fernanda Costa-Svedman – fernanda.costa-svedman@regionstockholm.se

Rita Hutyra-Gram – rita.hutyragram@ki.se

Standard Operating Procedure (SOP)

Processing of Urine Samples for the BioBladder Study

1. Collected Midstream Sample

A midstream urine sample is obtained from the patient.

2. Processing and Storage

- Transfer 30 mL of urine into two 15 mL Falcon tubes and seal with a cap.
- Centrifuge at 700 × g for 5 minutes.
- Carefully transfer the supernatant into two new 15 mL Falcon tubes.
- Seal the tubes, label them, and freeze at –80 °C.
- Until freezing, store samples in a refrigerator (4 °C).