**STANDARD OPERATING PROCEDURE**

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<th>Prostate Tissue Collection and Sampling</th>
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Electronic Filename: PCBN.SOP02.v1.4 Prostate Tissue Collection and Sampling
1. PURPOSE

To describe the procedure for the collection and transfer of prostate specimens from the Operating Room (OR) Suite to the JHU Urology and NYU pathology laboratories. To facilitate safe and efficient processing and sampling of prostate tissue with high integrity and quality. To describe the procedure for requisitioning paraffin blocks from pathology archives.

2. RESPONSIBILITIES

*Authorized personnel* collecting and sampling prostate tissue must ensure that:

- all tissue sampling procedures are followed correctly
- all tissue samples are adequately coded during processing
- all documentation is completed, and accurate records maintained on all samples

3. HEALTH AND SAFETY

Personnel carrying out this procedure must maintain safe working practices and observe all relevant Environmental Health and Safety (EH&S) guidelines. This includes the appropriate use of Personal Protective Equipment (PPE), and the procedures for waste disposal, disinfection & spill clean-up and biosafety.

4. EQUIPMENT AND MATERIALS

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<td>Containers (Isopentane, ice)</td>
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<td>Cryostat, Chucks, Freezing Bars</td>
<td>Cryomolds</td>
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<td>Dewar</td>
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<td>Paint brush</td>
<td>Liquid Nitrogen</td>
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<td>PPE (gloves, lab coat/gown, eye/face shield)</td>
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<tr>
<td>Sterile Forceps</td>
<td>Marking pencil and pens</td>
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<td>Sterile Surgical Handle</td>
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5. PROCEDURES

5.1 Sample Selection

- Specimens for procurement are selected based on site-specific criteria.

**Johns Hopkins University Network Site**
- Specimens for harvest are selected based on meeting one of four criteria:
  1. If Gleason patterns 4 or 5 adenocarcinoma were found on biopsies
  2. If 3 biopsy cores were positive for adenocarcinoma
  3. If one core was more than 50% positive for adenocarcinoma
  4. If the patient is involved with a clinical trial that requires tissue to be harvested

*NOTE:* At the JHU Network Site, there is in place an IRB-approved waiver of consent to harvest prostate cancer and non-neoplastic tissues from radical prostatectomy specimens.

**New York University Network Site**
- Specimens for harvest are selected based on meeting one criterion – obtaining appropriate patient informed consent.

5.2 Transporting of tissue from the OR to Pathology

- Upon resection of the prostate, the OR nurse alerts the biorepository designate.

**Designate:** the person responsible for obtaining the sample – Research Coordinator

- Immediately after being notified by OR, the Research Coordinator retrieves the specimen from the OR Suite.

*NOTE:* Specimens are retrieved within 5-10 minutes of alert.

- The Research Coordinator delivers the specimen to the Pathology Department (accessioning desk).

*NOTE:* When transporting tissue, keep the prostate cool by placing the specimen on ice. The prostate should never be allowed to come into contact with ice or ice water.

It is recommended that tissue collections kits be prepared in advance to minimize the total procurement process – the time the tissue is subjected to ischemic conditions is related to the quality of the specimen procured which can affect its scientific utility.

- Once at Pathology, the Surgical Gross Room Accessioning desk accessions the prostate specimen for processing and/or procurement.
NOTE: Delivery and accessioning is expedited to ensure procurement begins at the earliest possible time. Only specimens that meet the site-specific selection criteria are harvested. All specimens are generally processed/frozen within 20-30mins from resection.

5.3 Pathology Processing / Grossing

- Once accessioned, the prostate specimen is handled according to conventional pathology techniques for the respective institution.

NOTE: At NYU, processing is performed by the pathologist unless the responsibility is delegated to a pathology assistant or designated tumor repository technician. At JHU, processing is performed by the Research Coordinator who has been trained under the supervision of the pathologist.

*Johns Hopkins University Network Site*

*When the specimen meets the criteria for harvesting of fresh frozen tissue described above, the case is harvested by “Pinning and Punching”.*

- Specimens are grossed using a modified method (Srigley) to allow procurement.
- The prostate is weighed, measured and inked.
- The apex is amputated at its distal portion, further sectioned and submitted on edge as the apical margin.
- The bladder neck margin is shaved and submitted on edge.
- The remaining prostate is serially sectioned in the transverse plane into 2-3mm thick slices.

NOTE: During the procedure of serial sectioning slices are taken such that they maintain a continuous margin around them.

- Tissue and prostatic fluid is harvested (refer to sections 5.4 – 5.6 below).
- The case is turned over to the surgical pathology assistant (pathology assistants, or PA) at this point who is briefed regarding as to the status of the case.
- The remaining (non-frozen) pinned prostate tissue is floated in formalin for 1-5 hours prior to further sectioning.
- The prostate is then submitted entirely from apex to base in a manner that is amendable to 3D reconstruction of the entire prostate.

*New York University Network Site*

- Specimens are grossed using a modified method (Srigley) to allow procurement.
- The prostate is weighed, measured and inked.
The apex is amputated at its distal portion, further sectioned and submitted on edge as the apical margin.

The bladder neck margin is shaved and submitted on edge.

The remaining prostate is serially sectioned in the coronal plane into 2-3mm thick slices.

The capsule portion of slices II and IV are removed in one of two methods: the “orange peel” or the “controlled capsule excision” method and submitted for pathological diagnosis to ensure adequate evaluation of margins and stage.

**Orange peel method:** Peel off of capsule by removing the peripheral 1-5 mm of tissue using a scalpel or scissor. The removed sheet of capsule tissue is then separated into right and left sides, then cut into 2-3mm strips and submitted for diagnosis.

**Controlled capsule excision:** After sectioning the prostate into several sagittal slices from apex to base, the slices are further sectioned by removal of the peripheral 2-3mm “capsule” from the alternate slices selected for procurement.

### 5.4 Prostatic / Seminal Vesicle Fluid Procurement

**NOTE:** Harvesting prostatic fluid is performed by the pathologist unless the responsibility is delegated to a pathology assistant or designated Research Coordinator.

*Johns Hopkins University Network Site*

For all resected prostate specimens, an attempt is made to collect prostatic fluid in a manner that is as sterile as possible.

- Label a 1.5 ml Sterile Cryo-vial with O-Ring is labeled with the Brady Urological Institute Tumor Bank Number, the Surgical Pathology number and “PF” (tissue designation).
- Wearing gloves, the specimen container is opened, and the prostate is poured out onto the bench top that is covered with clean under pad.

**NOTE:** Care is taken to avoid contamination by not touching the distal urethral margin (DUM).

- Before weighing the prostate and prior to inking (refer to 5.3 above), express the prostatic fluid. The prostate is manipulated with pressure and massaging by both holding in hands and further manipulation on the bench top.
- Collect the fluid as it exudes from the DUM in the pre-labeled tube. Replace the top on the cryovial,
- Snap-freeze in liquid nitrogen.

**NOTE:** When snap-freezing, hold the vial perpendicular in a Dewar filled with liquid nitrogen to insure the fluid freezes in the bottom of the tube.
• Transfer the tubes from the Dewar into cardboard boxes (in sequential order) and store in the designated -80C freezer.

New York University Network Site
• After weighing the prostate and prior to inking (refer to 5.3 above), gently massage the prostate and seminal vesicle (SV).
• Truncate a portion of the seminal vesicles.
• Express the seminal vesicle fluid. Manipulate the SV by massage, then gently squeeze to express the fluid.
• Collect the fluid as it exudes from the SV in a labeled tube. Replace the top on the cryovial,
• Snap-freeze in a small container containing pre-cooled isopentane.

NOTE: When snap-freezing, hold the vial perpendicular to ensure the fluid freezes in the bottom of the tube.

• Transfer the tubes from the Dewar into cardboard boxes (in sequential order) and store in the designated -80C freezer.

5.5 Seminal Vesicle (SV) Procurement

NOTE: Harvesting seminal vesicle (SV) tissue is performed by the pathologist unless the responsibility is delegated to a pathology assistant or designated Research Coordinator.

• After weighing the prostate and prior to inking (refer to 5.3 above), truncate a portion of the seminal vesicles.

Johns Hopkins University Network Site
• Transfer the truncated SV tips into a labeled vial.
• Snap-freeze the vials containing prostatic fluid and SV in liquid nitrogen.

NOTE: The vials should be labeled with the surgical pathology number; the tissue designation, the Tissue Bank Number.

New York University Network Site
• Place the truncated SV tips into a cryomold labeled RT + LT SV.
• Fill the cryomold with OCT. Ensure that the tissue is covered by OCT.
• Use forceps or transfer pipette to orient tissue and remove air bubbles.
• Snap-freeze the vial of prostatic fluid and SV in a small container containing pre-cooled isopentane.
• Place the frozen vial/cryomold in a zip-lock specimen bag labeled with a unique specimen identifier.
5.6 Fresh-Frozen Prostate Tissue Procurement

- Specimens are harvested according to techniques for the respective institution.

  NOTE: Harvesting fresh-frozen prostate tissue is performed by the pathologist unless the responsibility is delegated to a pathology assistant or designated Research Coordinator.

*Johns Hopkins University Network Site*

- Select tissue for harvest, tumor and adjacent normal.

  NOTE: Palpable or visible tumor areas are targeted, in addition to several areas from each side of the peripheral zone

- Take a punch biopsy (0.5 – 1.0 cm) sample of the tumor and normal prostate tissue (total 2-5 tissue samples). The samples are labeled with designations of A, B, C, etc.

  NOTE: After punching, specimens are pinned out on paraffin wax and a diagram is made in the Prostate Tumor Bank Log Book where the diagram indicates the location of each sample.

- Tissue is placed on OCT on a plastic transfer strip and slide off onto the cryofreezing bar that is located in the bottom of the cryostat.

- Slowly and carefully guide into the desired position.

- Fill the wells so that a meniscus of OCT is bulging above the brim.

  NOTE: Approximate freezing times at -24C using a cold chuck ranges between 20-60sec depending on well depth. Cut two frozen sections for H&E staining per biopsy.

- Press the Weighted cold chuck over each well which acts as a heat sink.

  NOTE: The frozen sections should be labeled with the surgical pathology number; the tissue designation, the Tissue Bank Number,. One H&E goes to the resident assigned to the case while the second is kept by the biorepository.

- After sectioning, remove the frozen block from the chuck by warming. Ensure the tissue does not thaw.

- Using a clean blade cut away excess frozen OCT to reduce the size of the frozen block such that it fits into a pre-labeled vial with tight fitting lid.

  NOTE: The vials should be labeled with a unique Tissue Bank Number, Surgical Specimen Number, and Block Designation. No patient identifiers are placed on the vials.

- Place the vial with the specimen into a Dewar containing liquid nitrogen.
At the end of each day, transfer vials into cardboard boxes (in sequential order) on dry ice, into a designated -80C freezer.

**New York University Network Site**
- Select tissue for harvest - the inside part (capsule removed) of the alternate slices (slices II, IV)
- Depending on prostate size, bisect slice II or slice into quadrants.
- Embed each half/quarter into a cryomold, labeled 2 RT Prost and 2 LT Prost, or 2 RT Post, 2 RT Ant, 2 LT Post and 2 LT Ant.
- Cut slice IV into quadrants.
- Place the posterior portions into cryomolds, labeled 4 RT Post and 4 LT Post. Submit the anterior portions for pathological examination.
- Fill each cryomold with OCT. Ensure that the tissue is covered by OCT.
- Use forceps or transfer pipette to orient tissue and remove air bubbles.
- Place the cryomolds in a small container containing pre-cooled isopentane and submerge the moulds until the OCT is completely frozen (white and solid).
- Place each frozen cryomold in a zip-lock specimen bag (as per 5.4) labeled with a unique specimen identifier.
- Transfer the bag on dry ice for temporary storage at -80C.

NOTE: H&E characterization of bank samples is batched, using one of two methods: frozen section characterization (final confirmation) or H&E evaluation of alternate mirror slice (alternate surrogate for prioritizing frozen section characterization).

- After sectioning, remove the frozen block from the chuck by warming. Ensure the tissue does not thaw.
- Place the frozen block in aluminum foil and transfer into cardboard boxes (in sequential order) on dry ice, into a designated -80C freezer or vapor phase LN.
5.7 Formalin Fixed Paraffin Embedded (FFPE) Prostate Tissue Procurement

- After fresh tissue harvest, the remainder of the prostate is submitted for pathology examination.

**Johns Hopkins University Network Site**
- For prostates that are not subjected to sectioning for harvesting of fresh frozen tissue, the prostate is injected with formalin, as described in Ruijter ET al, Journal of Pathology 1997;183(3):369-375, “Rapid microwave-stimulated fixation of entire prostatectomy specimens.
- Rest the prostate for 30mins.
- Place in the microwave for 6 minutes.

  NOTE: Prostates weighting more than 50 grams sit for 30 minutes, get micro waved for 6 minutes sit for 15 minutes and then get micro waved for an additional 3 and one half minutes. The microwave settings are 50 degrees C and 450 Watts.
- Gross and process according to conventional pathology techniques for the respective institution for pathological examination.
- After pathological examination and reporting, FFPE tissue is archived.

  NOTE: Only FFPE tissue for cases identified for specific projects are requisitioned from archives.

**New York University Network Site**
- The prostate is injected with formalin.
- Gross and process according to conventional pathology techniques for the respective institution for pathological examination.
- After pathological examination and reporting, FFPE tissue is archived.

  NOTE: Representative FFPE tissue for all cases with appropriate consent are requisitioned from archives.

**FFPE Tissue Requisition**
- Representative samples of FFPE prostate tissue used for diagnosis are identified (from final histopathology and pathology review).
- These blocks (and corresponding H&E slides) are pulled from archives, and stored in temperature controlled archives at the biorepository laboratory.
- All blocks removed are replaced with a card indicating that they are being held by the biorepository.