SOP 03: P-MRI Biopsy Procedure and Tumor Tissue Storage

1.0 Biopsy Procedure Overview

The mpMRI-ultrasound fusion guided transrectal prostate biopsy procedure will conform to each established local institutional protocol. Use of local anesthesia is recommended. Prostate volume, presence of lesions, and digital rectal examination findings are required at each biopsy. Diagnostic biopsy schemes should incorporate at least 12 standard template biopsies. Each region of interest identified on MRI that is at least PIRADs 3 will be segmented and biopsied with at least 2 needle samples. Each ROI and core will be registered individually. Nitrocellulose touch preps (tissue prints) will be collected from each biopsy core. If at least one PIRADS 3 or higher lesion seen the "targeted" biopsy must be performed on fusion platform (eg MIMS, UroNav, Artemis) so as to track the needle core. If not performed, a deviation form is required. Even if there is there are no region of interest on MRI, the "targeted" biopsy should be performed on fusion platform (eg MIMS, UroNav, Artemis) so as to track the needle core. Needle core location information should be saved for upload to JPL.

2.0 Biopsy Procedure

The collection of prostate biopsy tissue is an important and mandatory part of this trial. Biopsy cores will be procured as per protocol and tissue prints will be processed promptly. Cores will be placed in standard formalin for processing either at centralized pathology (University of Michigan Site ID 918) or local pathology department. After clinical pathologic interpretation, the remaining formalin-fixed, paraffin-embedded (FFPE) blocks (or up to 10 unstained slides with tumor) will be sequestered and banked as part of the tumor bank. Tissue Prints will go to University of Miami (Site ID 311) and FFPE blocks (or up to 10 unstained slides with tumor) kept locally until an NCI designated Repository is identified. The tissue may be used by researchers now or in the future to better understand the nature of prostate cancer and how patients respond to treatment. Patients will not be identified by name. The only identification of tissue will be unique Specimen ID affixed by the site using the DMCC provided labels. Radiology reports (redacted) should be supplied as part of the supporting documentation required for this study and uploaded through the Data Transfer system in VSIMS using the Event Identifier. Pathology reports should be kept locally and made available to the DMCC upon request for auditing purposes. Patients on whom tissue is collected will be aware of this retrieval and will have given their consent to have their biopsy tissue-prints, RNA and DNA and biopsy tissue blocks (or up to 10 unstained slides with tumor) stored for purpose of research at University of Miami and an NCI designated repository.

Biopsy tissue will be treated as follows: A tissue print will be obtained from each core and each core will be oriented with a visible dot of permanent marker to mark the needle-point end of both the core and corresponding tissue print. Tissue prints will be immediately snap frozen and tissue cores immersed in neutral buffered formalin. Each MRI targeted core and each standard core will be submitted in a separate formalin jar as a separate specimen. Tissue prints are sent to University of Miami (Site ID 311).

Formalin-fixed, paraffin-embedded (FFPE) biopsy tissues: Pathologists will evaluate each biopsy core individually as part of the diagnostic pathology report. FFPE tissue from each biopsy/selected biopsies will be collected from the MRI targeted and standard diagnostic biopsy cores. FFPE blocks (or up to 10 unstained slides with tumor) will be sequestered and stored at the NCI designated biorepository. Sites should submit the case with the highest Gleason Score and highest Volume for submission to the tissue repository. If not available, then submit the case with the highest Gleason. If not available, submit the case with the highest volume.

Central review: Upon request from the DMCC some cases may be randomly selected for central pathology review at University of Michigan (Site ID 918). If selected, then all biopsy tissue should be sent for review. The tissue will be returned upon completion.

Nitrocellulose biopsy tissue prints:

Biopsy tissue prints will be used for RNA and DNA tissue biomarkers studies. **The collection of nitrocellulose tissue prints is optional.** The RNA and DNA tissue biomarkers will be analyzed in association with clinical information and common data elements appropriate for evaluation of prediction of cancer and prediction of >Gleason sum 6 cancer when used in combination with mpMRI imaging.

Nitrocellulose touch preps (tissue prints) will be obtained from each biopsy core during mpMRI-US fusion biopsy procedure during the transfer of the tissue core from the cutting needle to the formalin fix jar (see figure B). The contact

made between the fresh tissue biopsy and the nitrocellulose is sufficient to transfer enough cells to the membrane for the purposes of the EDRN studies without compromising the core for surgical pathology. A permanent marker dot for orientation is applied to the tissue print to allow for registration of tissue biomarker, histology and mpMRI imaging data. Tissue prints are then immediately snap frozen and the tissue cores submersed in neutral buffered formalin.

All tissue print collection supplies will be provided by the EDRN and prepared by the Gaston laboratory at University of Miami. Labels and worksheets will be provided by the DMCC.

3.0 Biopsy Tissue Print Collection Materials and Procedures

Collection Materials: Prostate biopsy equipment (cutting needle, imaging equipment, formalin jars etc) and personal protective supplies (gloves, gowns etc) as per routine procedure at the clinical site. Biopsy tissue print collection kit will be supplied by the EDRN (see Figure A).

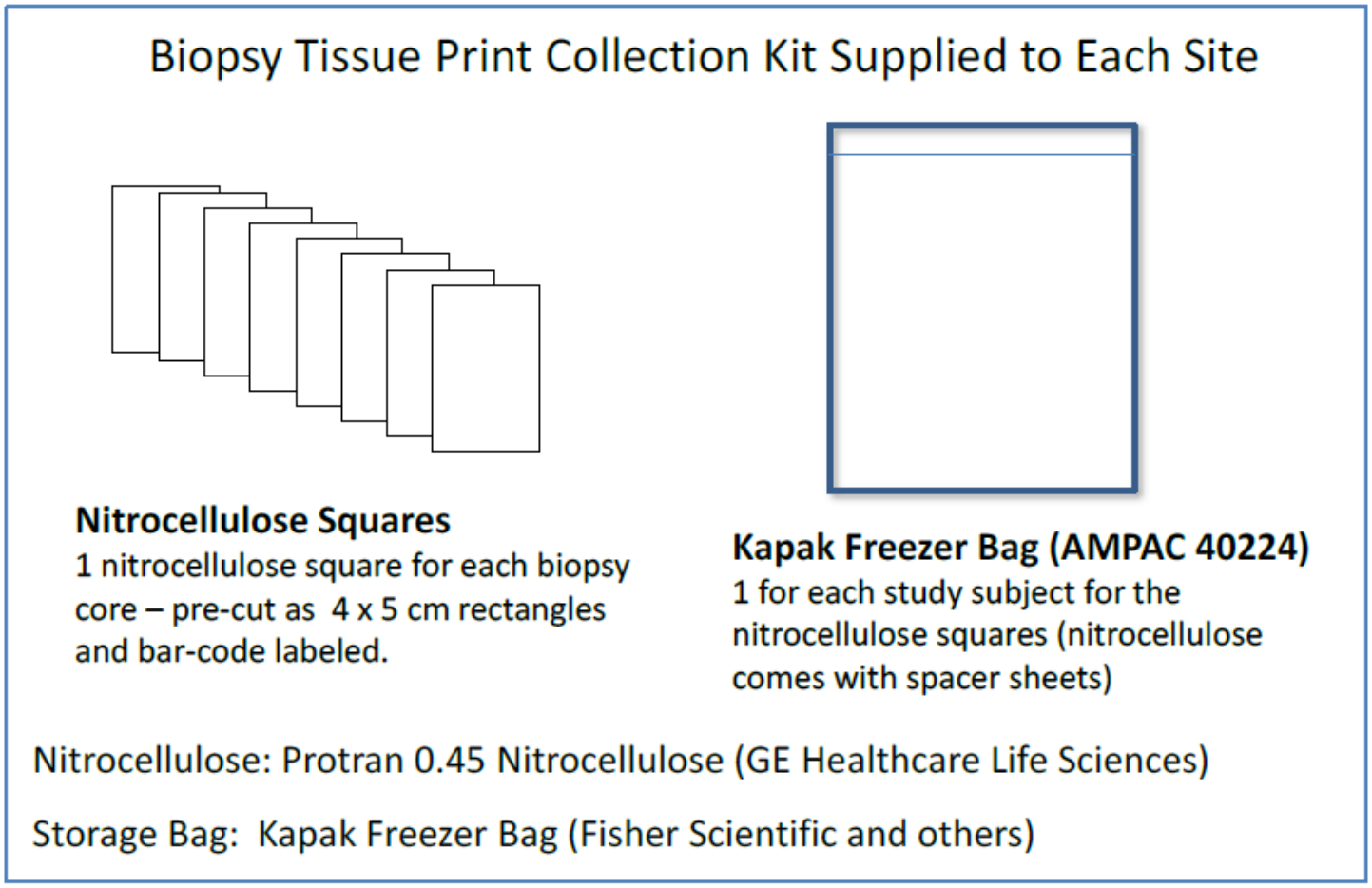
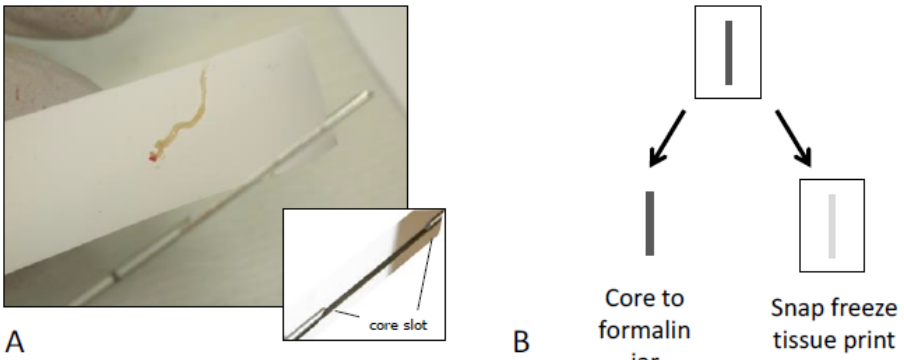


Figure A. Biopsy tissue print collection kit (supplied to study site by EDRN)

3.1 Collection of Biopsy Tissue Prints:

Each tissue core is transferred to the nitrocellulose to make a "touch prep" (see Figure B). The print and core are then marked with a permanent marker dot at the needle-point end (Figure C). The core is then transferred to the formalin jar as usual and the nitrocellulose tissue print is snap frozen on dry ice or on a freezer brick.



A. Photo of transfer of biopsy core directly from cutting needle to nitrocellulose square.
B. The tissue core is transferred to a formalin jar as usual. The nitrocellulose tissue print is snap frozen on dry ice or on -80 freezer brick .

Figure B. Biopsy tissue print collection – transfer of core from cutting needle to the nitrocellulose square

End-labeled tissue print
snap frozen on dry ice

The biopsy tissue print and core are end-labeled at the needle-point end with a permanent marker

FFPE end-labeled tissue core

Figure C. End labeling of tissue print with permanent marker

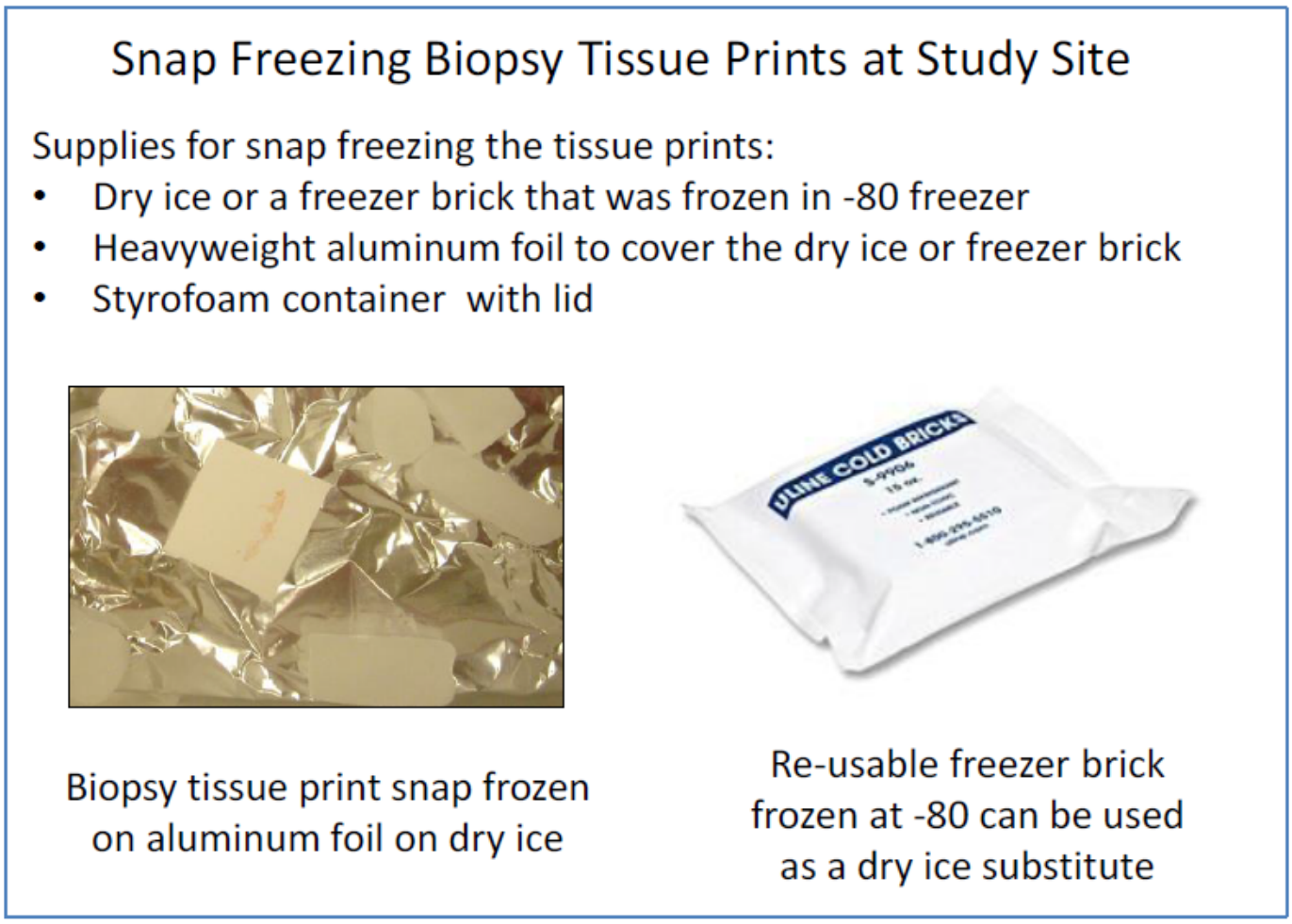


Figure D. Tissue prints are snap frozen on dry ice or on a freezer brick that was frozen in -80^{0}C freezer. Use heavyweight aluminum foil to cover the dry ice or freezer brick. Freezing is usually done in a styrofoam container with a lid. Each tissue print is snap-frozen as it is collected during the biopsy procedure When the biopsy procedure is completed, the frozen tissue prints are placed into a Kapak storage bag for frozen storage (see 11.6.3.3).

Note that variations on the tissue printing procedure have proven to be effective. At most clinical sites, a single biopsy cutting needle is used for each patient biopsy procedure and as each core is obtained the tissue is transferred from the needle to a formalin jar on a gauze pad, blue sponge or some other support. Depending on local preference, the nitrocellulose square can be used in place of the standard transfer support (see figure B) or in combination with the standard transfer support (ie the nitrocellulose square can be used to pick up of core from the gauze pad). On boarding training will be provided by Dr. Sandra Gaston.

3.2 Storing biopsy tissue prints at study site prior to shipment to the processing lab:

The nitrocellulose squares come with protective blue spacer paper. All of the snap frozen tissue prints from a single biopsy procedure are placed into the Kapak storage bag (included in the tissue print kit Figure A) with the protective blue spacer paper between the white nitrocellulose sheets. The Kapak bag is then stapled shut. Bar code ID stickers are attached to the outside of the Kapak bag. The tissue prints in the Kapak bag are then stored flat in a -80^{0}C freezer until shipment until being shipped to the processing lab on dry ice.

3.3 Quality assurance: RNA and DNA:

RNA and DNA from the first 5 sets of biopsy tissue prints submitted by each study site will be analyzed in detail to document the transfer of cells and the integrity of the nucleic acids prepared from the tissue prints. Additional sets of tissue prints will be analyzed in detail at established intervals to monitor specimen quality.

3.4 Tracking IDs of biopsy cores, tissue prints and images:

The bar code IDs on the tissue prints, Kapak bag, tissue cores and mpMRI images are entered into VSIMS or LabCAS, accordingly.

4.0 Radical Prostatectomy Overview

If the participant undergoes radical prostatectomy upon progression, prostate tissue will be acquired as FFPE blocks (or up to 10 unstained slides with tumor) for tissue banking. Both malignant and benign tissue will be sampled.

1. Pathologist will systematically and uniformly section entire prostate according to the institution's procedure. Since it is not always standard to section the entire prostate, the coordinator must inform appropriate staff so that this happens.
2. The Pathologist at each study site must review all slides and reports generated from study participants. The fact that this has happened will be documented in VSIMS.
3. University of Michigan or the local Pathologist at each study site will select at least one representative FFPE block containing tumor tissue and one representative FFPE block containing normal tissue from tissue that remains after pathological diagnosis, and these blocks (or up to 10 unstained slides with tumor) will be sequestered for PMRI study. Blocks (or up to 10 unstained slides with tumor) will remain in pathology department files for at least 6 months after surgery. Blocks (or up to 10 unstained slides with tumor) will be pulled to be sequestered for PMRI study in batch mode but must be done at minimum every 6 months. The blocks (or 10 unstained slides with tumor) will be stored at the NCI designated repository.
4. Coordinator will obtain pathology report and use it to complete the PMRI Prostatectomy Form in VSIMS. It is recommended that the hardcopy form be completed prior to key entry in VSIMS.
5. FFPE radical prostatectomy tissue will be banked in the tissue bank at the NCI designated repository.

5.0 FFPE Tissue Blocks and Slides from Biopsies or Radical Prostatectomy

Tissue Blocks and/or up to 10 slides are to be entered into VSIMS using the DMCC worksheets provided (examples below). Tissue should be stored ambient at room temperature. Select biopsy cores to be submitted based off the following hierarchy:

1. Highest Gleason Score and Highest Volume
2. Highest Gleason only regardless of Volume
3. Highest Volume regardless of Gleason

P-MRI FFPE BIOPSY Tissue Block and Slides

Version 2.0
05-17-2019
page 1 of 1

Site Participant ID (affix label):

Staff ID: _____ Site ID: _____

Biopsy Date: ___ - ___ - _____

Tissue Block must be entered in VSIMS as a "Parent" specimen in order for tisue slides to be entered (even if no block will be sent).

☐ FFPE Biopsy Block **418108712**

Cabinet #	Box #	Slot # (1-100)

Pathology Report identifier (CDE 2709): as listed on pathology report (e.g. A, B, C, T1C1, T1C2, or HGPIN T1C1, HGPIN T1C2, etc.) ☐

☒ *if collected*	Cabinet #	Box #	Slot # (1-100)	Staining? (H/E, Unstained, Other)	SOP followed?	Defective?	Reason Defective (broken, lost, large portion missing, significant sectioning artifacts)
☐ 418108712-1					Y / N / Unk	Y / N	
☐ 418108712-2					Y / N / Unk	Y / N	
☐ 418108712-3					Y / N / Unk	Y / N	
☐ 418108712-4					Y / N / Unk	Y / N	
☐ 418108712-5					Y / N / Unk	Y / N	
☐ 418108712-6					Y / N / Unk	Y / N	
☐ 418108712-7					Y / N / Unk	Y / N	
☐ 418108712-8					Y / N / Unk	Y / N	
☐ 418108712-9					Y / N / Unk	Y / N	
☐ 418108712-10					Y / N / Unk	Y / N	

P-MRI FFPE PROSTATECTOMY Tissue Block and Slides

Version 2.0
05-17-2019
page 1 of 1

Site Participant ID (affix label):

Staff ID: _____ Site ID: _____

Tissue Block must be entered in VSIMS as a "Parent" specimen in order for tisue slides to be entered (even if no block will be sent).

☐ FFPE Prostatectomy Block **418108712**

Cabinet #	Box #	Slot # (1-100)

Pathology Report identifier (CDE 2709): as listed on pathology report (e.g. A, B, C, T1C1, T1C2, or HGPIN T1C1, HGPIN T1C2, etc.) ☐

☒ *if collected*	Cabinet #	Box #	Slot # (1-100)	Staining? (H/E, Unstained, Other)	SOP followed?	Defective?	Reason Defective (broken, lost, large portion missing, significant sectioning artifacts)
☐ 418108712-1					Y / N / Unk	Y / N	
☐ 418108712-2					Y / N / Unk	Y / N	
☐ 418108712-3					Y / N / Unk	Y / N	
☐ 418108712-4					Y / N / Unk	Y / N	
☐ 418108712-5					Y / N / Unk	Y / N	
☐ 418108712-6					Y / N / Unk	Y / N	
☐ 418108712-7					Y / N / Unk	Y / N	
☐ 418108712-8					Y / N / Unk	Y / N	
☐ 418108712-9					Y / N / Unk	Y / N	
☐ 418108712-10					Y / N / Unk	Y / N	