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Biospecimen Collection from Patient Biopsies

I. Purpose

The purpose of this document is to describe the procedure for collecting and processing tissues from patient biopsies. These patients can be enrolled in a variety of projects, including POG (Personalized Onco Genomics), PROFYLE (Precision Oncology for Young People) and PANGEN (Prospectively Defining Metastatic Pancreatic Ductal Adenocarcinoma Subtypes by Comprehensive Genomic Analysis).

II. Scope

All procedures are applicable to the BCGSC Biospecimen Core Group.

III. Policy

This procedure will be controlled under the policies of the Genome Sciences Centre, as outlined in the Genome Sciences Centre High Throughput Production Quality Manual (QM.0001). Do not copy or alter this document. To obtain a copy see a Quality Systems associate.

IV. Responsibility

It is the responsibility of all personnel performing this procedure to follow the current protocol. It is the responsibility of the Biospecimen Core Group Leader to ensure personnel are trained in all aspects of this protocol. It is the responsibility of Quality Systems Team to audit this procedure for compliance and maintain control of this procedure.

V. References

Document Title	Document Number
Genome Sciences Centre Occupational Health and Safety Manual	N/A

VI. Related Documents

Document Title	Document Number
Biopsy Summary Sheet	BSCore_Forms.0021
Biopsy Sectioning Checklist	BSCore_Forms.0023
FFPE Requisition Form	Generated by CTAG/Histology Lab

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VII. Safety

All Laboratory Safety procedures will be complied with during this procedure. The required personal protective equipment includes a laboratory coat and gloves, as well as protective eyewear during biopsy procedure. In case of a lung biopsy (Bronchoscopy or CT guided), a N95 mask should be worn. See the safety data sheet (SDS) for additional information.

It is strongly recommended that any person coming in direct contact with bodily fluids, such as blood, are vaccinated against the following diseases: Measles Mumps Rubella and Hepatitis B. Familiarize yourself with the Genome Sciences Centre Occupational Health and Safety Manual before handling any blood samples. Ensure you are familiar with the sections outlined below:

- Chapter 31 – Laboratory Biosafety
- Appendix 3 – BCCA Blood and Body Fluid Exposure Protocol
- Appendix 4 – PHSA Blood and Body Fluid Exposure FAQ
- Appendix 23 – Emergency Response Guide to Human Tissue, Blood or Bodily Fluid Exposure

VIII. Materials and Equipment

Name	Supplier	Number	Model or Catalogue #	
RNase Zap	Ambion Inc	AM9780		✓
80% Ethanol	In House	N/A	N/A	N/A
C-fold paper towel	Fisher Scientific	06-666-114		✓
Bench Coat	Fisher Scientific	14666300		✓
Aluminum cryo-mold holder plate	In House	N/A		N/A
Ziplock bags	VWR	CA40000-101		✓
Absorbent Bench Underpads	VWR	56617-014		✓
Dry Ice	In House	N/A		N/A
Styrofoam box	In House	N/A		N/A
Forceps, sharp	VWR	47743-174		✓
Forceps, blunt	Fisher Scientific	08-887		✓
Conical Bottom Centrifuge Bottle, PS, 175mL	Thermo Scientific	3145-0175		✓
OCT compound	CW Stores	31331		✓
Cryo-molds (Tissue-Tek, 25x20x5mm)	Cedarlane Lab	62534-25		✓

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Name	Supplier	Number	Model or Catalogue #
Sterile saline vials	Trudell Medical Marketing	H0120039	✓
T36	VWR	CA26200-152	✓
Alcohol wipes	VWR	2910-110	✓
Biohazard bags	VWR	56766-372	✓
Absorbent pads	Praxair	MVE10777446	✓
Disposable gowns	Global Industrial	WBB895245	✓
Masks	Fisher Scientific	19166634	✓
Sterile gloves, S and M	SafeTouch	1172B, 1137C	✓
Petri dishes	Fisher Scientific	08-757-168	✓
Cryo-tubes, 5mL	Thermo Scientific	12590001	✓
Disposable scalpels	Fisher Scientific	12460451	✓
Non-adherent pads (Curad)	Medline	NON25710	✓
Formalin Specimen Jar (10% NBF)	Leica	380075424	✓
2D barcode labels	Microsystems	296352422	✓
Small Plate labels	Fisher Scientific	296352422	✓
Pen	Ryzex	84068	✓
Marker	Staples	N/A	N/A
Custom spinning device (for OCT)	Staples	N/A	N/A
	In House	N/A	N/A

IX. Procedure

Background: Different types of biopsy procedures may be performed in order to obtain tissue specimens from patients. Described in this protocol are the most common:

1) Needle core biopsies: Tissue samples are obtained via a biopsy needle. The needle core samples are then embedded with the OCT compound into cryo-molds and frozen on dry ice. We request 4-6 needle core samples from an 18G biopsy needle, ideally, to maximize the likelihood of obtaining sufficient tissue and tumour content for successful genomic analysis. If the needle size is smaller than 18G, then additional cores, if possible, can be helpful to increase the amount of tissue obtained.

2) Larger fresh tissue biopsies (e.g. bite or excisional biopsies): These tissue samples may be snap frozen or embedded with OCT in a cryo-mold based on the size of the tissue. The cryo-molds have dimensions of 24mm long, 19mm wide, and 5mm deep. If the tissue removed is smaller than approximately 2x3x10mm, these samples are preferred to be embedded with OCT

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in a cryo-mold. Any tissues larger than 2x3x10mm can either be cut into smaller pieces and embedded with OCT in a cryo-mold or placed in a cryo-tube and frozen on dry ice.

Regardless of biopsy type, it is important that the specimen is frozen as soon as possible in order to maintain nucleic acid integrity and gene expression profile. One to two technicians may attend a biopsy procedure in order to ensure proper handling and freezing of the tissue specimen. If it is not possible to freeze the tissue immediately store the specimen on wet ice until it can be frozen.

At several steps during a biopsy procedure the attending technician(s) may be required to make a judgment call for example whether to embed or flash freeze a sample.

Attending biopsy procedures involves interactions with patients and being exposed to sensitive patient information. Ensure that patient confidentiality is a priority at all times.

1. Preparations Prior to a Biopsy

These preparations are to be done within a day of the scheduled procedure.

1.1. Determine when and where the collection is taking place and who will be attending. This is typically scheduled by the APC and/or PC.

1.1.1. Review the Biopsy Summary Sheet (BSCore_Forms.0021) to ensure that all of the appropriate fields are completed in order to confirm the patient details in Step 2.10.1 (e.g. Project ID, BCCA ID, initials, gender) and to determine the location of the biopsy. This form can be found in the appropriate locked cabinet.

1.1.2. Place the folder containing the Biopsy Summary Sheet (BSCore_Forms.0021) in the top zippered pouch of the Medpac bag.

1.2. Preparation of Cryo-molds

Note 1: Cryo-molds should be prepared with a very thin layer of OCT compound on the bottom of the cryo-mold **prior** to the biopsy procedure. A custom (made in-house) spinning device is used to spread the OCT on the bottom of the cryo-mold (see Figure 1). This can also be done by hand (see Step 1.2.7.2).

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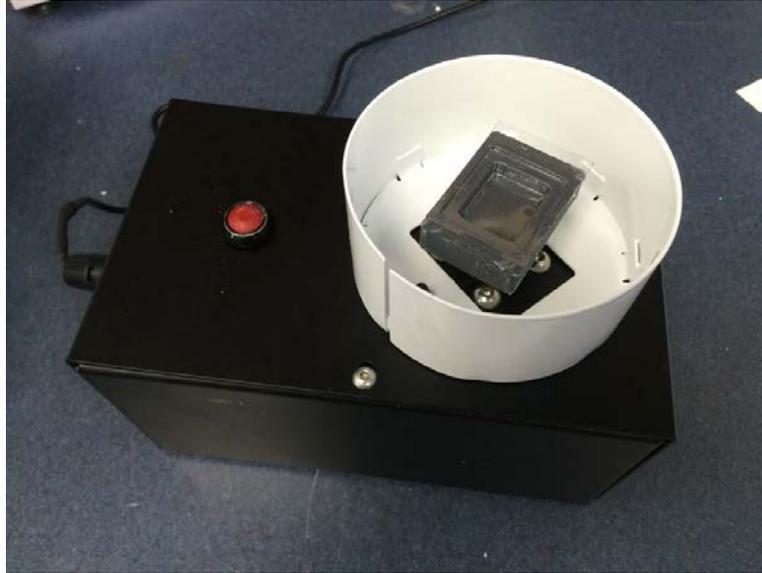


Figure 1: Custom spinning device

Note 2: A custom (made in-house) aluminum plate with 6 cut-outs for cryo-molds is used. This plate ensures fast freezing of the embedded tissue and results in uniformly frozen molds (see Figure 2). When handling the plate when cold, make sure to only hold the plastic insert (black or white) as the aluminum will burn your skin when cold.

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Figure 2: Custom aluminum cryo-mold holder plate

- 1.2.1. Prepare 6 cryo-mold labels per Biopsy case. Each cryo-mold label will display the Project ID plus mold ID, e.g. POG ID Bx A. For 6 labels the mold IDs will be: Bx A, Bx B, Bx C, Bx D, Bx F, and Bx G. Bx E is reserved for the sample placed in formalin in Step 2.11.3. To prepare these labels, refer to Appendix A. Wearing a lab coat and gloves, prepare the work area. Ensure the work area is clean and clear. Apply RNase Zap to the bench and wipe down with a large Kimwipe. Next apply 80% ethanol to the bench and wipe down with a large Kimwipe.
- 1.2.2. Affix new bench coat to the cleaned work surface.
- 1.2.3. One aluminum plate is required per biopsy case. Each plate holds 6 cryo-molds. The aluminum plate must be cleaned prior to use. To do this, apply RNase Zap to the plate and wipe with a large Kimwipe. Next apply 80% ethanol to the plate and wipe with a large Kimwipe.
- 1.2.4. Retrieve a Styrofoam box with a biohazard label from a -80°C freezer. Ensure sufficient dry ice (~2/3 full). Place the aluminum plate as level as possible on the dry ice to pre-chill.

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- 1.2.5. Clean your gloves by applying RNase Zap and rub your hands together. Next apply 80% ethanol to your gloves and rub your hands together. Use a Kimwipe to wipe away any excess moisture.
 - 1.2.6. Lay out 6 cryo-molds on the bench coat. Label each cryo-mold with the labels prepared in Step 1.2.1.
 - 1.2.7. A thin layer of OCT compound must be spread uniformly across the bottom of the cryo-mold. This can be done with or without a spinning device. If a spinning device is available to use, proceed to Step 1.2.7.1. If no spinning device is available proceed to Step 1.2.7.2.
 - 1.2.7.1. Place a small drop of OCT compound (~0.5cm diameter) in the centre of the cryo-mold. Place the cryo-mold in the spinning device. Make sure the mold is seated level and all four corners are pushed down. Spin for ~5 seconds or until OCT has spread out over the entire surface of the cryo-mold. Make sure there are no bubbles.
 - 1.2.7.2. Place a drop of OCT compound in the corner of the cryo-mold and use a spreading device (the edge of a cryo-mold can be cut and used for this purpose) to evenly distribute a thin layer of the OCT across the bottom of the mold.
 - 1.2.8. Place the cryo-mold in one of the 6 cut-outs of the pre-chilled aluminum plate.
 - 1.2.9. Repeat Steps 1.2.7 and 1.2.8 to prepare the remaining cryo-molds.
 - 1.2.10. When all 6 cryo-molds have been prepared, place the aluminum plate with the cryo-molds in a ziplock bag to protect from contaminants. Place the bag and plate back on the dry ice in the Styrofoam box.
 - 1.2.11. Place the Styrofoam box (with the aluminum plate with cryo-molds) back in the -80°C freezer until ready to go to the biopsy.
- 1.3. Check the Medpac bag contains the following items:
1. RNase Zap
 2. C-fold Paper towels
 3. Absorbent Bench Underpads (~3)

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4. Kimwipes
5. Sterile forceps, sharp and blunt: 1 each
6. OCT compound: Minimum ½ full bottle
7. Sterile saline vials
8. Container (conical bottom centrifuge bottle, 175mL) with ~5mL T36 (for dirty forceps)
9. Alcohol wipes
10. Biohazard bags
11. Absorbent pads
12. Disposable lab gowns (~3)
13. Masks (surgical and N95)
14. Sterile gloves (S and M)
15. Sterile Petri dishes
16. Cryo-tubes, 5mL
17. Formalin jar (10% NBF)
18. Disposable scalpels
19. Disposable blades
20. Disposable transfer pipettes
21. Sterile Non-adherent pads (Curad or Telfa)
22. Empty cryo-molds
23. Blank labels (2D and small plate)
24. Pens and markers
25. Clock
26. Light
27. Safety glasses
28. Pre-filled requisition forms for CTAG

2. Attending a Biopsy

- 2.1. Retrieve the Styrofoam box with the prepared cryo-molds from the -80°C freezer. Place the Styrofoam box in a carry bag. Retrieve the Medpac bag with all of the supplies.

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- 2.2. Depart the lab in time to arrive at the biopsy location. 10-20 minutes prior to the scheduled biopsy time should be sufficient depending on the location.
- 2.3. Once at the location, notify the technician or nurse that you are there to collect tissue specimens from a biopsy procedure. Wait in the waiting area until you are given permission to enter the procedure room to set up.
- 2.4. If possible, confirm with the doctor who is performing the biopsy the desired number of samples for the project (e.g. 4-6 needle core, 18G, samples). You might not be able to procure the preferred amount of tissue since patient safety is the priority: The doctor will decide how many samples can be safely provided.
- 2.5. Wear a clean disposable gown, or provided gown, gloves and protective eyewear.
 - 2.5.1. If the procedure takes place in an OR room, you may need to put on scrubs, shoe covers, hair cover and a mask. These will be provided. Ask a nurse if you need any assistance.
 - 2.5.2. In case the procedure is a lung biopsy (Bronchoscopy or CT guided), a N95 mask that has been fitted should be worn.
- 2.6. Set up in the designated area (ask the technician or nurse for a suitable area if needed).
 - 2.6.1. Apply RNase Zap to the bench and wipe down with a paper towel. Next clean the area with an alcohol wipe.
 - 2.6.2. Spread out an absorbent bench underpad.
 - 2.6.3. Retrieve the following from the Medpac bag:
 1. OCT compound bottle
 2. Forceps (sharp or blunt)
 3. Saline vial
 4. RNase Zap
 5. Styrofoam box with aluminum plate with prepared cryo-molds
 6. Formalin jar (10% NBF)
 7. Disposable scalpel (optional)
 8. Petri dish(es)

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9. Clock
 10. Pen
 11. Light
 12. Sterile Non-adherent Curad pad
 13. File folder containing Biopsy Summary Sheet (BSCore_Forms.0021).
-
- 2.7. If working in pairs, one person will be responsible for tracking the cold ischemic times and entering information on the Biopsy Summary Sheet (BSCore_Forms.0021) and the other person will be processing the tissue (embedding or snap freezing).
 - 2.8. Clean your gloves by applying RNase Zap and rubbing your hands together.
 - 2.9. Remove the aluminum cryo-mold plate from the ziplock bag and place it on the dry ice as level as possible.
 - 2.10. Completing the Biopsy & Sectioning Summary Sheet (BSCore_Forms.0021):
 - 2.10.1. Before the biopsy starts, confirm the patient information on the Biopsy Summary Sheet (BSCore_Forms.0021) matches with the patient information available in the procedure room: e.g. from a Requisition form if possible or any other documentation available (e.g. a printed label). Ask the technician or nurse if necessary. Place a check mark beside each field that is able to be verified. In some cases a PHN number is used instead of the BCCA ID. If this is the case, write down the PHN number on the Biopsy sheet to verify later with a Project Manager.
 - 2.10.2. Record the name of the doctor (often already pre-filled) who will be performing the biopsy in the Performed by section.
 - 2.10.3. Record name(s) of the (GSC) staff attending the biopsy in the Attending section.
 - 2.10.4. Record Biopsy Needle Type (e.g. Bard or QuickCore) and Gauge if applicable (only applicable at needle core biopsies) in the Biopsy Notes section. Ask the technician or nurse if necessary.

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2.10.5. Confirm the Tissue site. If the tissue site being biopsied differs from the site provided record the new tissue site.

2.10.6. When the biopsy procedure starts, record the time each biopsy is taken in the 'Time Tissue Removed' section. Assign each biopsy a number.

2.10.7. Record the time when the tissue is placed on dry ice (either embedded in a cryo-mold or placed in a cryo-tube) in the 'Time Tissue Placed on Dry Ice' section.

2.10.8. Record which biopsies go into which molds, e.g. Biopsy 1 goes in cryo-mold A, Biopsy 2 goes in cryo-mold B or Biopsy 1 and 2 are going both in cryo-mold A.

In case a large piece of tissue is divided between cryo-molds or cryo-tubes, record this (e.g. Biopsy 1 into cryo-tube A and B)

2.10.9. Record any other details about the biopsy in the Biopsy Notes section.

2.10.9.1. Record when one or more biopsies are from a different location/lesion than the others, as these biopsy tissues will have different OS names and should not be pooled during subsequent steps.

2.11. Processing tissues

Note: In cases where the tissues are needle cores or relatively small pieces of tissue, you will embed the tissue with OCT compound in a prepared cryo-mold. Depending on the size of the needle cores you'll embed one (typical) or multiple (if small) per cryo-mold, see Step 2.11.1. In cases where the tissues are larger pieces (e.g. bite, excisions) they can be (cut into smaller pieces and) embedded in OCT compound or snap frozen in cryo-tubes, see Step 2.11.2.

2.11.1. Needle core tissues:

2.11.1.1. Tissues can be transferred to you in various ways. It will depend on the doctor's preference or tissue quality and/or quantity.

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- 2.11.1.1.1. If on a sterile non-adherent pad (e.g. Telfa or Curad): if pad has not been pre-soaked with saline by the doctor, place a few drops of saline on the needle core tissue to avoid tissue sticking to the pad.
- 2.11.1.1.2. If washed with saline into a sterile Petri dish: move needle core tissue away from the saline solution and/or transfer to a non-adherent pad to dry the tissue.
- 2.11.1.2. Examine the needle core tissue(s) you have received and decide how you are going to embed (e.g. single core or combine multiple cores in one cryo-mold).
- 2.11.1.3. Retrieve a prepared cryo-mold from the aluminum plate in the Styrofoam box. Defrost the cryo-mold by holding a finger along the bottom (this is to avoid freeze/thaw of the tissue). Place on the work surface close to tissue.
- 2.11.1.4. The structure and consistency of the tissue can vary, from very solid and rigid to very soft and fragile. Be sure to handle the tissue as gently as possible to ensure the cell structure is not (or minimally) damaged.
- 2.11.1.5. With forceps (typically a sharp forceps is used for needle core tissues, but a blunt forceps could be used if core is very large or the consistency requires it) gently grasp the needle core sample at one end and transfer the tissue to the prepared labeled cryo-mold. The tissue should be placed into the centre of the cassette and laid flat and straight onto the thin layer of OCT compound.

Note: It is essential that the tissue core is laid flat, and is as parallel to the bottom of the cryo-mold as possible. This is necessary to facilitate subsequent cryo-sectioning of the tissue, so that each section will pass across the full length of the tissue, maximizing

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the amount of tissue in each section and minimizing the number of sections required to exhaust the tissue.

Figure 3 shows frozen OCT molds with embedded needle core tissues. The molds have been inverted so that the tissue cores are visible. Note that each core is laid flat and straight, and they are within ~ 1mm of the bottom of the cryo-mold.



Figure 3: Embedded needle core tissues in OCT

2.11.1.6. Fill the cryo-mold with OCT compound. Avoid introducing air bubbles adjacent to the tissue. If several small pieces of a needle core sample have been placed together into a mold, quickly snap freeze the tissues in the mold prior to filling the mold with OCT compound. This will prevent the tissue pieces from moving apart.

2.11.1.7. Place the cryo-mold onto the aluminum plate to freeze. Make sure the aluminum plate is as level as possible to ensure a level OCT surface in the cryo-molds.

2.11.2. Larger tissue pieces (e.g. bite, excisions):

2.11.2.1. Tissue can be transferred to you in various ways: In a container with sterile saline solution, in a Petri dish, or on a non-adherent pad are some examples.

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- 2.11.2.2. If the tissue is in a container with saline solution, transfer it to a sterile non-adherent pad to remove the excess moisture.
- 2.11.2.3. If the tissue is covered in blood, transfer to a sterile non-adherent pad and rinse with sterile saline.
- 2.11.2.4. Examine the tissue(s) you have received and decide how you are going to process them.
- 2.11.2.4.1. If tissues are small (approximately less than 2x3x10mm) and soft, embed in OCT compound in a prepared cryo-mold – see Step 2.11.1.
- 2.11.2.4.2. If tissues are larger than approximately 2x3x10mm, they can be cut up in smaller pieces and embedded in OCT compound in prepared cryo-molds.
- 2.11.2.4.2.1. Retrieve a disposable scalpel from the Medical bag.
- 2.11.2.4.2.2. Transfer the tissue to a clean Petri dish. Each tissue will be different in size and shape: try to cut it into thin, even slices of ~ 2-3mm thickness.
- 2.11.2.4.2.3. Transfer the cut pieces with forceps to labeled cryo-molds, lay as flat as possible on the bottom of the mold and embed in OCT compound.
- 2.11.2.4.3. If tissue is very large, it needs to be cut up in smaller pieces and snap frozen in separate cryo-tubes.

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- 2.11.2.4.3.1. Retrieve a disposable scalpel from the Medical bag.
- 2.11.2.4.3.2. Transfer the tissue to a clean Petri dish. Each tissue will be different in size and shape: try to cut it in even sized pieces with a maximum thickness of 3 mm.
- 2.11.2.4.3.3. Transfer the smaller pieces with forceps to labeled cryo-tubes.
- 2.11.3. After receiving 4 cryo-molds (or cryo-tubes) worth of tissue, the '5th' tissue removed will be placed in a formalin jar to have a FFPE block made. Put this sample aside (in Petri dish or on non-adherent pad) until all processing of all other tissues has been completed, then put the tissue into the formalin jar. Label the jar with Project ID and 'BxE'. Place the jar inside a Biohazard bag.
- 2.11.4. In case more than 6 cryo-molds are needed to embed all the tissues received, prepare extra cryo-mold(s) as follows:
 - 2.11.4.1. Retrieve empty cryo-mold(s) and blank label(s) from the Medpac bag and hand label the cryo-mold(s).
 - 2.11.4.2. With the OCT compound bottle 'paint' on a thin layer on the bottom of the cryo-mold. Try to avoid bubbles.
 - 2.11.4.3. Embed the tissue – see Steps 2.11.1.4 - 2.11.1.7.
- 2.11.5. Once the molds or tissues are frozen, place a Biohazard bag labeled with the Project ID on the dry ice and transfer the samples to the bag. Keep the bag on dry ice in the Styrofoam box.

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2.12. Clean up

2.12.1. Place the used forceps in the container with T36.

2.12.2. Dispose of any used items in the biohazard waste container in the procedure room.

2.12.3. Put all other items back into the medical bag.

2.12.4. Place the aluminum plate in the outside sleeve of the ziplock bag and place in the outside pocket of the carry bag.

2.12.5. Place the Styrofoam box into the carry bag.

2.13. Carry the cryo-molds or cryo-tubes on the dry ice to CTAG. Transfer the biohazard bag containing the tissue to the designated CTAG -80°C freezer.

2.14. If a formalin sample was collected, fill out a FFPE Requisition Form:

2.14.1. Enter Project ID plus BxE ('POGXXXX BxE'), and BCCA ID in the 'List of Sample ID's' section.

2.14.2. Enter the Date submitted and the name of the GSC technician dropping off the sample.

2.14.3. Place the form in the outside sleeve of the Biohazard bag containing the formalin jar and place in the designated CTAG drop off location.

2.15. Enter how many molds or tissues were collected at the biopsy in the 'CTAG' calendar, located in the CTAG/Histology lab.

3. Return to the GSC

3.1. Restock the Medical bag

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- 3.1.1. Place the container with the dirty forceps onto the lab bench in the container labeled 'For Autoclaving'. Fill a clean container with ~5ml of T36, place the container in a Biohazard bag with an absorbent pad, and place it into the Medpac bag.
- 3.1.2. Place new forceps (blunt or sharp, depending on what was used) in the Medical bag.
- 3.1.3. Add an absorbent bench underpad to the ziplock bag so there are at least 3 in the bag.
- 3.1.4. Replace or top up any other used supplies (e.g. replace the formalin jar in a biohazard bag if it was used).
- 3.1.5. Check if any other supplies are running low and replace as required (e.g. check the volume remaining of the OCT compound).
- 3.2. Place the folder with the Biopsy Summary Sheet (BSCore_Forms.0021) in the designated drawer in the locked cabinet.
- 3.3. Fill out the Biopsy Sectioning Checklist (BSCore_Forms_0023) with the following information:
 - 3.3.1. Project ID on both sides of the form.
 - 3.3.2. Record how many tissues (specify molds or tubes) were received.
 - 3.3.3. Record where the samples are stored.
- 3.4. To prepare for cryosectioning of these samples (i.e. label RLT+ tubes, pre-fill Project Sectioning Tracking sheets and Chain of Custody forms) refer to "Preparing Cryosections for H&E Staining and Nucleic Acid Extraction" (BSCore.0010).

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Appendix A

LIMS Protocol for Generating OCT Mold Labels

1. Generating Files and Printing

- 1.1. Log in the LIMS database by entering your User name and your Password. The Optional Startup Settings will default to the “Biospecimen_Core” Department and the “5th Floor Printers – Biospecimen Core” Printer Group.
- 1.2. Under the Lab tab select Project home (e.g. POG Home). Complete the fields of the Project home page Printer File as follows:
 - 1.2.1. Project Number: Enter only the Project ID number. e.g. 1234 (for POG) or 00423 (for PROFYLE).
 - 1.2.2. Number of Cassettes: Enter the number of OCT molds. e.g. 6.
 - 1.2.3. Highest RLT Section Number per Cassette: Number of sections per mold. e.g.30
 - 1.2.4. Date: Enter the date of the biopsy.
- 1.3. Click Generate Printer File.
- 1.4. Under OCT Mold Label Printer File, select the Printer from the drop down list and click Print Customized Barcodes.