Standard Operating Procedure (SOP) for Sectioning Frozen Tissue Samples in Histology

I. SCOPE AND PURPOSE

Fixation and heat involved in routine histologic processing can inactivate many antigens and genetic material within the tissue, therefore frozen sections must be used. This procedure is specific for the preparation of interval sections for H&E staining and scrolls for gene sequencing.

This procedure applies to all trained BCR laboratory personnel. The purpose of this Standard Operating Procedure is to establish a procedure for the Biospecimen Core Resource (BCR) to embed and section frozen tissues for pathology review.

See SOP H010, "Receiving Tissue in Histology from Logistics", SOP H011, "Returning Tissue to Logistics", and SOP A005 "LabVantage User Manual" for tissue transfer to and from Logistics.

II. PROCEDURE

A. Safety Procedures

1. Research Institute policy Research-(SAFETY)-(005), "Microtome and Tissue Sectioning" is used for safe operation of the microtome.
2. Use appropriate knife safety procedures: remove blades after use and dispose of in sharps container per SOP H007 "Microtome or Cryostat Blade and Razor Blade Disposal". Put blade safety cover on when not sectioning.
3. Cryostat and razor blades are very sharp! Wear cut resistant gloves under nitrile gloves at all times. Always lock cryostat handle before putting hands into the blade holder area. Always wear cut resistant gloves, nitrile gloves, and lab coat when handling blades which have been exposed to unfixed tissue. Never leave blades lying around where someone may accidently cut themselves.
4. Always remove knife or place knife guard on before reaching into chamber!
5. LOCK hand wheel when working with hands inside the cryostat.
6. Follow universal precautions: Wear gloves, mask, and gown when handling unfixed tissue.
7. Use caution when working with liquid nitrogen and dry ice. Skin exposure to these can cause burns. Wear appropriate gloves and gown when removing samples from liquid nitrogen vapors. If working with liquid nitrogen liquid (as opposed to vapor) use gloves, gown, and safety goggles or face shield per SOP BPC-SAF-004 Compressed Gases Hazards and Safety.
8. Ethanol is flammable. Store properly in flammable cabinet. Use gloves and lab coat when working with ethanol.
9. Read and know all SDS sheets for each chemical being used. Wear solvent resistant gloves (e.g., nitrile) and a lab coat impervious to fluids at all times when using the stainer.
10. All spills should be cleaned up and material disposed of in accordance to local, state, and federal laws.

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B. Quality Control

1. Check temperature of cryostat before beginning per SOP H008, "Monitoring Laboratory and Equipment Temperatures in the BCR Histology Lab".
2. Do not allow tissues to thaw at any time. If any temperature excursions occur, record the temperature and the length of time (if known) that the tissue was exposed to the temperature. This information should be recorded on the CDT sheet and in the comments sections of LabVantage.
3. Assure that the cryostat is clean and all instruments are wiped with 95% ethanol and gauze.
4. A new blade and razor blade is to be used with each specimen. Dispose of blades properly per SOP H007, "Microtome or Cryostat Blade and Razor Blade Disposal".
5. To avoid sample contamination, all gauze, gloves, and toothpicks used for a specimen are to be discarded and new gauze, gloves, and toothpicks are to be used for each sample.
6. The knife holder and all instruments (forceps, brushes, etc.) are to be cleaned with 95% ethanol between every sample.
7. Have **only one** sample at a time in the cryostat.
8. To avoid any mix up of samples tissue or slides the following steps are **required** when setting up to section the tissue:
   a. Place only one vial in the cryostat.
   b. Open vial and remove tissue for processing.
   c. Place empty vial on a small amount of dry ice in a box in the cryostat.
   d. Carefully match the slide to the vial label and place **only** the slide for that portion on the top of the cryostat.
   e. Do not place any other slides on the cryostat.
   f. Do not place any other vials in the cryostat.
   g. Check the slide against the vial before placing the section on the slide.
   h. Replace sectioned and inked tissue into the vial in the cryostat.
   i. Place slide into staining rack.
   j. Place completed portion on dry ice until it is returned to LN_2 vapors.

C. Required Equipment, Supplies, and Reagents

1. Equipment
   a. Cryostat
   b. Cryostat chuck
   c. Anti- roll plate (optional)
   d. cryocart
2. Supplies
   a. Apex glass slides, blue 1x3x1.0mm charged- Leica cat# 3800082
   b. MX35 low-profile, disposable – Fisher cat#3052835
   c. Coverslips- Mercedes Medical cat# CAS942450
   d. Micro-Mount mounting medium- Leica cat# 01731
   e. Single-edge Razor Blades- Fisher cat# 9412071
   f. Forceps- Fisher cat#08-953G

g. Camel-Hair brushes- Fisher cat# 03-670
h. Toothpicks
i. 4x4 Gauze Sponges- Cardinal KC9134B

3. Reagents
   a. 95% Ethanol- Fisher cat# 22032600
   b. Optimum Cutting Temperature (OCT) compound, Tissue-Tek- Fisher cat# 1437365 or Neg50 –Fisher cat#22046512
   c. Tissue-Marking dye-Cancer Diagnostics cat#-0727-2

D. Specimen Information

1. Type: Snap frozen, unfixed tissues stored on LN_2 vapor.
2. Handling Conditions: Store on LN_2 vapor until used. Transfer to dry ice. Keep sample in cryostat until sectioned and then replace to dry ice for transfer to long term storage in LN_2 vapor phase. Do not allow tissues to thaw at any time.
3. Sample Preparation: Most samples should be snap-frozen and stored in cryovials in liquid nitrogen vapor. Some samples may already be in OCT.

E. Sectioning Frozen Tissue

1. Prior to initiation of cutting tissue, ensure the cryostat is clean per SOP H002, "Cryostat Cleaning and Decontamination". Between cuttings, ensure it is free of debris and is wiped with 95% ethanol on gauze and dried with clean gauze.
2. Samples are received in Histology per SOP A005 "LabVantage User Manual" and SOP H010, "Receiving Tissue in Histology from Logistics". Slides and labels are created for each sample as directed by the submitting institution or project protocol (refer to SOP A005 for labeling procedure.)
3. When ready to process samples, take one sample from the transfer box and place into small dry ice container.
4. Check sample ID number with custody domain transfer (CDT) paperwork and initial paperwork. Take slides that match the sample.
5. Place vial with portion (e.g., 01) into cryostat. Allow tissue to equilibrate to -20° C (about 2-3 minutes).
6. Open vial and locate dot of OCT on tissue which indicates tissue orientation (unless the vial is labeled "unmarked.") For all samples the OCT dot is on the top of the portion (e.g., portion 01, 02, 03 etc.)
7. Mount tissue on chuck (specimen holder) using very small amount of OCT. Place the side <u>without</u> OCT onto the chuck so the top surface of the sample will be cut. (**Exception**: All portions will be cut from surface with OCT dot on it EXCEPT portion 3 for tissues of <u>normal</u> orientation (NOT flat) <u>tumors</u> (NOT controls). These will have a slide made from the side opposite the OCT dot.

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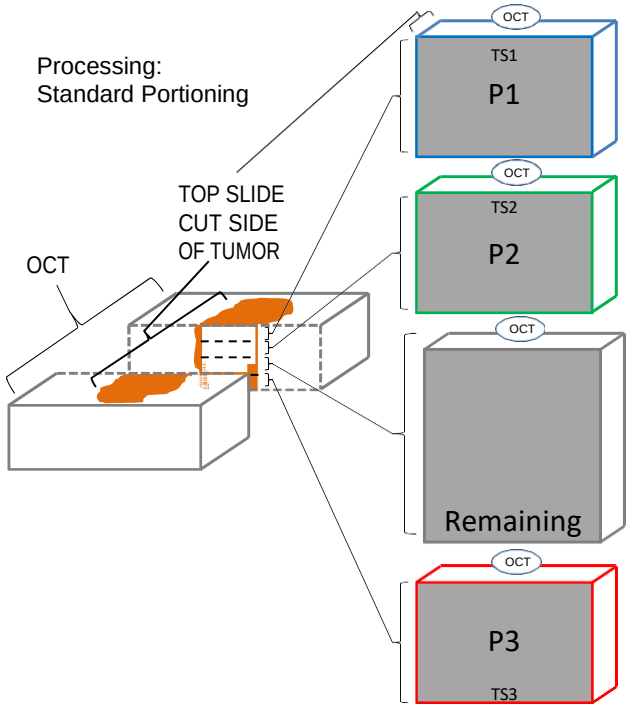

8. Allow OCT to harden. Apply a small amount of OCT to the top surface of the tissue with a toothpick. Try to place a small amount of OCT around the top edge.
9. Place new blade in holder and tighten down securely. Put chuck with tissue into holder and tighten down
10. Angle blade and specimen so block face and knife edge are parallel. Optimum angle is usually around 10 degrees but may vary.
11. Carefully advance knife to start taking sections. Do not face too far into the block - save as much tissue as possible for DNA and RNA extraction.
12. Take one or two 4 -6 micron sections and place on labeled slide for H&E staining. Center tissue sections in middle of slide (top to bottom, side to side).
13. Lock hand wheel and cover blade edge with roll plate. Remove chuck with tissue from the holder and place on gauze piece in cryostat. Use a new razor blade to cut the tissue off the chuck.
14. If samples require a section from the bottom of the sample. Proceed to section II.E. 15. If only a top section is required, proceed to section II.E.19. If a bottom section is requested but tissue portion is less than 5mm in thickness, do not cut a bottom slide. (Check all such portions with histology manager.) Proceed to section II.E.19.
15. If a slide was created for a bottom section, and it was not used, dispose of the unused slide in LabVantage and dispose of the physical glass slide in the broken glass container. Make comment on paperwork and also in LabVantage that portion is “top slide only”.
16. If the portion is greater than 5mm in thickness continue with Section II.E. 17. and cut a bottom slide.

17. Take a clean, precooled chuck and place a drop of OCT on it. Place removed tissue sample – cut side down – onto that drop of OCT. The bottom (OCT-embedded) side of the tissue should now be up.
18. Repeat Sections II.E. 8-13.
19. Mark tissue bottom with a small dot of ink. Put ink on OCT near tissue but not on tissue.
20. Cut off as much OCT as possible with razor blade. Re-label with ink if necessary.
21. Used chucks are placed in approved disinfectant (e.g., 95% ethanol alcohol or 10% bleach solution).
22. Replace tissue into cryotube and put tube in small dry ice container. If there is a second portion (e.g., 02), remove it and place in the cryostat. Repeat sections II.E.4-15.
23. Stain slides per SOP H001, "Hematoxylin and Eosin (H&E) Frozen Section Stainer"
24. Return samples in tubes to original container. Make any comments on the CDT transfer request paperwork and in Lab Vantage.
25. All samples will be returned to LN_2 vapor storage when box is finished per SOP A005 "LabVantage User Manual" and BCR SOP H011, "Returning Tissue to Logistics".
26. Dispose of all blades per SOP H007, "Microtome or Cryostat Blade and Razor Blade Disposal"), gauze, gloves, and toothpicks. Clean cryostat and all instruments with 95% ethanol.

F. Interpretation, Analysis, Documentation

1. The H&E stained sections on slides are to be reviewed for stain and section quality. Ensure that sections do not have folds or tears and are not too thick or too thin. Slides with unacceptable appearance are recut.
   a. Sections should not fragment or split. If this occurs, temperature may be too cold. Warm chamber slightly to correct, usual temperature range is -20 to -35^{o}C. Do not go above -20^{o}C
   b. Sections should not collect on knife edge. If this occurs, temperature may be too warm. Lower chamber temperature and recut. Leica cryostat will go as low as -50^{o}C
   c. Fatty tissues may require very low temperatures (-35 to -50^{o}C) or slightly thicker sections (up to 8-10 microns).
2. Sectioning from each case should be documented on appropriate CDT paperwork and in LabVantage. Note any issues with the tissue and add tech initials.
3. Slides that pass QC should be released to Virtual Microscopy (VM). Slides that do not pass QC should be retained for recuts.

III. REFERENCES

A. BCR-REF-001, "BCR Acronym List"
B. BCR-SOP-A005 "LabVantage User Manual"
C. BCR-SOP-H001, "Hematoxylin and Eosin (H&E) Frozen Section Stainer"
D. BCR-SOP H002, "Cryostat Cleaning and Decontamination"
E. BCR-SOP-H007 "Microtome or Cryostat Blade and Razor Blade Disposal"

F. BCR-SOP-H008, "Monitoring Laboratory and Equipment Temperatures in the BCR Histology Lab"
G. BCR-SOP-H010, "Receiving Tissue in Histology from Logistics"
H. BCR-SOP-H011, "Returning Tissue to Logistics"
I. Research-(SAFETY)-(005), "Microtome and Tissue Sectioning"
J. Carson, F. L. (1996). *Histotechnology: A Self-Instructional Text* (2 ed.). ASCP Press.

IV. COMPREHENSIVE REVISION HISTORY

A. Changes made to Version 2, Effective Date **8/19/2016**
1. Made title not all capitalized
2. Made cryostat generic.
3. Added language to record temperature excursions
4. Slides and labels are created for samples as directed by submitting institution or project protocol

B. Changes made to Version 2, Effective Date **08/26/2014**
1. New format used
2. Minor word and grammatical changes made throughout
3. Updated title for clarification
4. Added reference to the Research Institute's new safety policy on microtome and tissue sectioning
5. The term Material Safety Data Sheet (MSDS) was updated to the current term of Safety Data Sheet (SDS)
6. Removed references to TCGA so that the procedure may be used for multiple projects
7. This SOP replaces the Histology Section of SOP LH003, "Sectioning and Portioning TCGA Frozen Tissue Samples"

C. Version 1, Effective Date 8/22/2012 - New

Signatures

Approved By: **Signature on file** **Date:** **Date on file**

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