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RECEIPT, OCT EMBEDDING AND HISTOLOGY QUALITY CONTROL OF BREAST BIOPSIES AND SURGICAL SPECIMENS

1. PURPOSE

The aim of this Standard Operating Procedure is to standardize the receipt, OCT embedding and sampling for histology quality control of breast needle core biopsies and surgical specimens.

2. SUPPLIES

Reception and rinsing of samples

- Sterile Transfer Pipets
- 15 mL Sterile Conical Tubes
- Sterile Petri Dish
- RNase-Free PBS (10x)
- RNase-Free Water
- Gloves
- Crushed Ice

Embedding in OCT and freezing

- Sterile Tweezers and Transfer Pipets
- Sterile Scalpel Blades
- Sterile Gauze
- Metal Beaker
- Thermos
- 2-Methyl Butane (Isopentane)
- Ethanol 70%
- Formalin 10%
- NaOH 1% (in RNAse free water)
- RNAse free water
- Cryomolds
- OCT
- Freezer Storage Boxes
- Ziplock Bags 2”x3”
- Disinfectant Solution
- Tissue Sampling and H/E Staining
- Gloves
- Knife for Microtome
- Thin-End Tweezers
- Sterile and RNAse-Free Conical Tubes (15mL)
- Dry ice

3. PRECAUTIONS

Gloves must be worn at all times when handling specimens. Tissue material is considered a biohazard and should be handled using universal precautions according to local Health and Safety rules.

Material used for rinsing and embedding (PBS aliquots, transfer pipettes, molds, OCT, Ziplock bags, gloves) should be stored separately from material used in the laboratory routine workflow (ie: placed in a clean plastic box or large ziplock bags) and dedicated to handle these specimens only.

Important note: Gloves should be changed frequently to eliminate sources of contamination.

4. WORKING PROCEDURES

4.1 Reception

- Upon arrival of the specimens, complete and follow instructions on the requisition form that is received with the biopsy samples to confirm their reception.

- Immediately place the frozen samples (liquid nitrogen and RNA later samples) at -80^{o}C, or proceed directly with the rinsing step.

4.2 Freezing media preparation

- Samples should be frozen in 2-Methyl butane (isopentane) refrigerated either with liquid nitrogen or dry ice. Let evaporate under the hood after use.

Pre-frigeration with liquid nitrogen:

- Fill a dedicated thermos about mid-way with liquid nitrogen. Fill the metal beaker about one third of the way with 2-Methyl butane (isopentane).

- With long tweezers, slowly lower the beaker in the liquid nitrogen and close the thermos until ready to freeze the samples.

OR

Pre-frigeration with dry ice:

- Put 2-Methyl butane in a large metal container or glass beaker and on dry ice. Fill the metal beaker about one third of the way with 2-Methyl butane and deposit it on dry ice.
- Put 2-Methyl butane in a large metal container and add dry ice in and let it cool.

Note: 2-Methyl butane in contact with liquid nitrogen polymerizes and causes toxic fumes. When solidified, evaporation of e-methyl butane should be done under a hood.

4.3 Rinsing

- Fill a bucket with dry ice.

- Open a new bottle of DEPC-treated water and prepare 5 mL of 1X PBS from the solution of 10X RNAse free PBS in a sterile conical tube using single-use sterile pipettes.

- Place the 1X PBS tube on dry ice to cold the solution. If frozen, it should be shaken until liquefied.

- With a sterile transfer pipet, remove the maximum of RNAlater from the sample tube, without allowing the biopsy to dry.

- Empty the contents of the transfer pipet in a sterile petri dish in order to ensure that a fragment of the biopsy was not aspirated.

- Using another pipet, place 2 mL of 1X PBS in the cryovial. Place the cryovial on the dry ice and gently roll the vial over the dry ice so as to rinse the biopsy

entirely with the PBS. Keep on dry ice 1 to 2 minutes, ensuring that the biopsy is well submerged in PBS.

- Repeat the rinsing step one more time, taking care to minimize contact with the biopsy when removing PBS.

- Proceed with the OCT embedding step.

4.4 OCT embedding

- Samples snap frozen need to be equilibrated at the bottom of the cryostat for at least 30 minutes to bring it at the cryostat temperature.

- Select the correct size of cryomold for the size of the specimen and mark the number of the specimen on the cryomold with a permanent marker.

- Put a beaker with 100% ethanol in the cryostat, let it cool down and put the tweezers in the ethanol.

- Wash the tweezers with RNAse away before touching the samples.

- Remove the biopsy from the cryovial after the last PBS rinse (in the case of RNA later samples).

- If the biopsy carrot is longer than 1cm, place it on a sterile Petri dish and divide the carrot in two pieces using a sterile scalpel.

- Put a little amount of OCT in the mold, place the biopsy flat at the bottom of the cryomold. If the carrot was cut, align the fragments parallel.

- Fill the cryomold to the top with OCT, taking care not to apply too much pressure when pouring the OCT so that the biopsy remains in place and bubbles do not form. (pour it from a corner of the mold to ensure minimal moves of carrot(s))

- Put back the tweezers in the ethanol and wash with RNAse Away between each sample.

4.5 Freezing of the OCT block

- Verify that the temperature of the 2-Methyl butane is adequate to permit rapid freezing of the sample. If the sample freezes too slowly, tissue architecture will be altered. Temperature is adequate if the 2-Methyl butane has begun to freeze on the bottom and sides of the beaker, ensuring sufficient solution to submerge the cryomold.

- Gently submerge the cryomold, making sure that it remains horizontal. It takes between 45-60 seconds to freeze. OCT must be completely opaque and hard.

- Remove the OCT block from 2-Methyl butane with tweezers and quickly dab excess e-Methyl butane with an absorbent paper or sterile gauze. Slide the specimen in a Ziploc bag labelled with the specimen number.

- Proceed to sampling of the biospecimen or store in a -80^{o}C freezer in a box identified for study patient specimens. Should sampling be performed immediately after embedding, cryostat temperature must be set at the appropriate temperature (according to the tissue type) at least 0.5h prior to sampling in the cryostat to obtain optimal cutting temperature.

Temperature of cutting according to tissue type

Tissue	**Temperature (^{o}C)**
Breast	-25 to -30

4.6 Sampling of the OCT block

Note: All the material used in this procedure will be refrigerated ahead of time and should be free from any aqueous solutions.

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Histology quality controls

- Proceed first with deep cleaning of the cryostat using 70 % ethanol only, mostly the area around the blade.
- Set cryostat temperature at the appropriate temperature according to the tissue type.
- If the blocks were stored store in a -80^{o}C freezer, samples should be brought from the freezer to the cryostat on dry ice. Place all blocks into the cryostat and let them sit there at least 30 minutes to reach appropriate cutting temperature. This step applies also to biopsies that have been just embedded.
- Clean cryostat blade, tweezers and brushes with ethanol 70 %. Allow excess ethanol to dry and place all instruments to cool down in the cryostat together with microtubes and sterile scalpel.
- With the scalpel, cut one corner of the OCT block as a reference.
- Using a drop of OCT, fix the block to the cryostat chuck (sample holder)
- Proceed gently with sectioning avoiding thick trimming.
- When the specimen starts to be available for the collection of a section, make one 4/5 microns cut and collect it on a slide for H&E staining. The section should be placed on the slide so that the cut mark in the OCT block goes on the upper right corner of the slides.
- Detach the block from the chuck and replace it in its mold. Place this one back in its Ziploc bag 2”x3”. Store on dry ice until it returns to the freezer.
- Store the OCT block in a -80^{o}C freezer in a box identified for study patient specimens. Slides should be fixed in 10% buffered formalin prior to H&E staining (Refer to CTRNet standard procedure #8.3.007 available at: http://www.ctrnet.ca/operating-procedures).
- Carefully clean with ethanol 70 % all instruments and cryostat cutting zone before sampling a new specimen. The blade that has been previously used must be cleaned and can be re-used in a new zone of its edge.

- Brushes will be decontaminated after these manipulations by washing in NaOH1% RNase-free solutions and then rinsed in RNase-free water, ethanol 70%. Repeat the washing twice. Brushes will be dried and stored in a clean Ziploc bag.
- Submit the H&E slides to the pathologists for histological quality control.
- The pathologists will indicate the following:

  Inside the whole sample:

  1. Proportion of the sample presenting tumour tissue (percentage)
  2. Proportion of the carrot presenting normal tissue (percentage)
  3. Quality control: estimated quality of nuclear morphological preservation. 1=poor preservation of chromatin, nuclear shape and distinction of mitotic figures such that accurate grading is not possible, 2=moderate preservation such that some nuclear detail and mitotic figures can only be distinguished with difficulty, 3=good to excellent preservation of nuclear features.

  (In all cases (1) + (2) should sum to a total of 100%)

  Inside the tumor zone:

  1. Proportion of necrosis (percentage)
  2. Proportion of cellularity (percentage)
  3. Proportion of stroma (percentage)
  4. Level of inflammatory cell infiltration using the scale: 0 (none), 1 (1-20%), 2 (21-50%), 3 (51-100%)

  (In all cases (1) + (2) + (3) should sum to a total of 100%)
- Should the carrot present heterogeneity, the pathologist will annotate the slide with **a fine permanent pen** to indicate where the block will be sectioned.

4.7 Processing of the OCT block

- Prepare a bucket with dry ice.
- Proceed first with deep cleaning of the cryostat using ethanol 70 % only, mostly the area around the blade.
- Set cryostat temperature at the appropriate temperature according to the tissue type.
- Bring the OCT block from the freezer to the cryostat on dry ice. Place all blocks into the cryostat and let them sit there at least 30 minutes to reach appropriate cutting temperature.
- Allow all instruments to cool down in the crysotat (microtubes, sterile, scalpel and tweezers.
- For biopsies containing more than 70% tumor, cut off the OCT surrounding the sample using a scalpel.

  For biopsies containing less than 70%, the block will be cut according to the pathologist's indications using a sterile scalpel blade. Two blocks should be obtained: one normal and one tumoral. These blocks should be stored separately and properly identified.
- Deposit the biopsy sample isolated from the block in an eppendorf tube and place it on the dry ice bucket.
- Label the tubes properly indicating if it is tumor or normal tissue.
- Transfer the eppendorf immediately to -80^{o}C in a freezer storage box.

5. ABBREVIATIONS/DEFINITIONS

Abbreviation	Description
OCT	Optimal cutting temperature.Embedding medium for tissues for histopathologic analysis
RNAse	Ribonuclease. A type of nuclease that catalyzes the degradation of RNA

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PBS	Phosphate buffered saline. A solution containing sodium chloride and sodium phosphate
RNAlater	RNA later. By stabilizing the RNA in tissue samples this reagent eliminates the need to immediately process tissue samples.