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Tissue Procurement: Cryopreservation with OCT Compound

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1 Works for me dx.doi.org/10.17504/protocols.io.6y7hfzn

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ABSTRACT

Specimens are frozen to provide a hardened matrix for sectioning and to preserve the morphological, biochemical, and immunological properties of cells and tissues. Freezing tissue specimens has the potential to eliminate many problems associated with standard practices of chemical fixation and paraffin or resin embedding. In practice however, freezing can dramatically alter the physical and chemical structure of cells and tissues and cause the formation of ice crystals. Cryoprotectant agents control the rate of cooling in order to limit the formation of ice crystals. Optimal Cutting Temperature (OCT) is a routinely used water-soluble glycol and resin cryoprotectant agent that provides an excellent specimen matrix for cryostat sectioning at temperatures of -10°C and below.

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MATERIALS TEXT

Supplies

1. Safety glasses or face shield
2. Disposable sterile gloves
3. Freezer-insulated gloves
4. Personal protective gear (eye glasses, face shield, lab coat)
5. Tissue Tec OCT cryo-compound at room temperature
6. Clamshells, pre-labeled with barcode (2d), age, sex, race of donor, weight of sample and anatomic site and tissue type
7. Dry ice transport container
8. Sharps biohazardous waste container

BEFORE STARTING

- Perform protocol on dry ice. Note: The vapor from dry ice obscures photos. The photos in this protocol were taken of the tissue on the bench top ONLY to depict how the tissue should look at each stage.
- The OCT compound is poured into a UV sterilized, bar coded clamshell.

Tissue Procurement: Cryopreservation

1 Procure anatomic site and tissue type per request, but sizes should be no larger than 0.4cm x 0.4cm x 0.3cm. OCT ratio should be 30:1 to properly infiltrate the tissue.

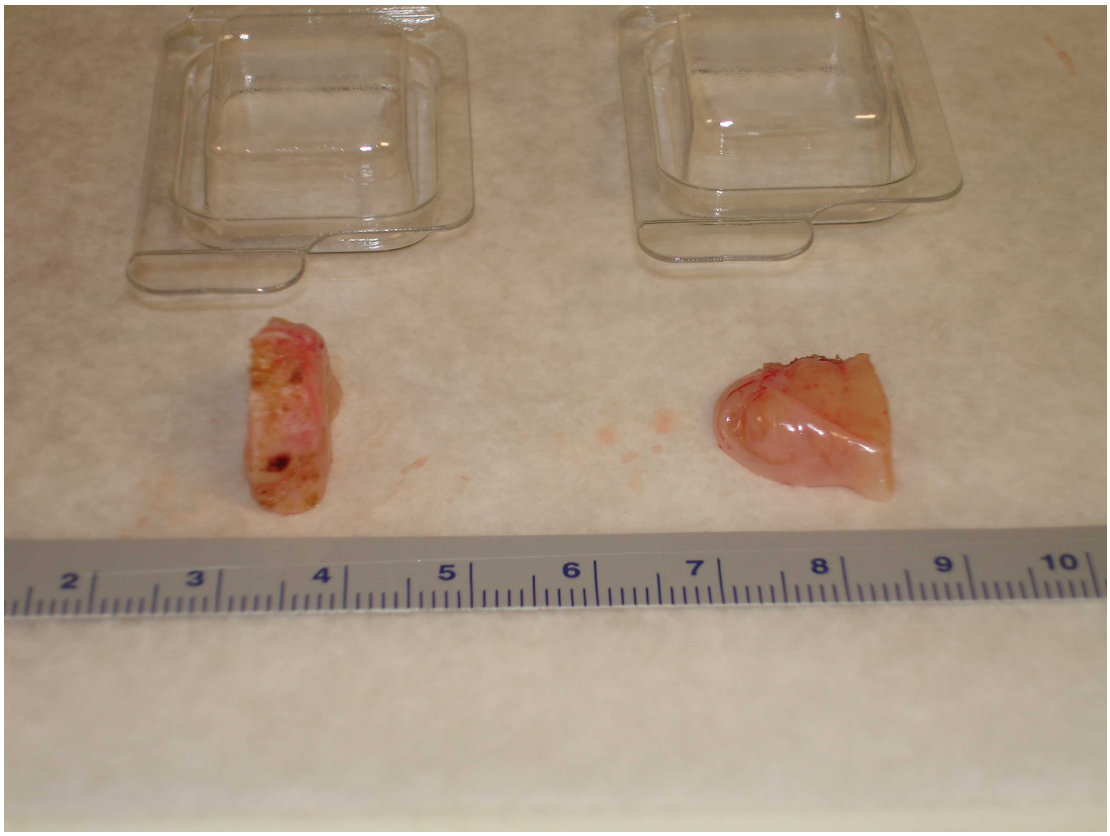

Figure 1. Cut tissue into approximately 1 cubic centimeter pieces.

2 Arrange the pre-labeled, UV sterilized clamshells on a block of dry ice to cool. Make sure the clamshells are evenly laid on the dry ice to allow the OCT to be evenly aliquoted.

3 Invert the OCT compound to ensure that no bubbles will be introduced in the media, as this will cause problems with the microtome (artifacts, shearing and samples not cutting cleaning or laying cleanly) and avoid uneven distribution.

4 Fill each clamshell with OCT by slowly and carefully filling the mold with an even layer, so that the OCT just touches all sides of the clamshell, just enough to OCT to cover all tissue. DO NOT allow the OCT to freeze completely white, but with sterile forceps or needle, transfer the specimen to the OCT-filled cryomold and gently submerge the tissue into media in the correct orientation and cover with additional OCT.

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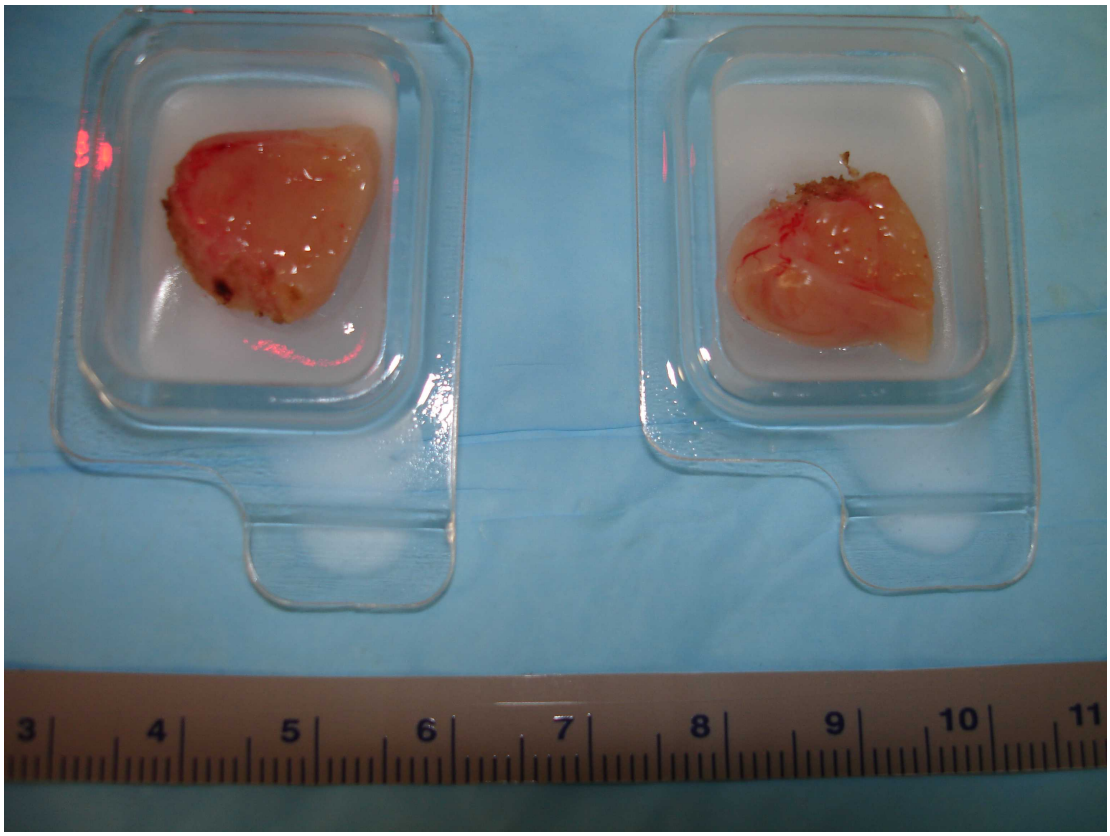

Figure 2. Place tissue in clamshell and fill clamshell with enough OCT to just cover the tissue.

5 Harden by placing back in the dry ice chest. DO NOT leave the specimen on dry ice for any longer than it takes to turn the completely white, as overcooling can cause cracking and brittleness, rendering the sample un-usable.

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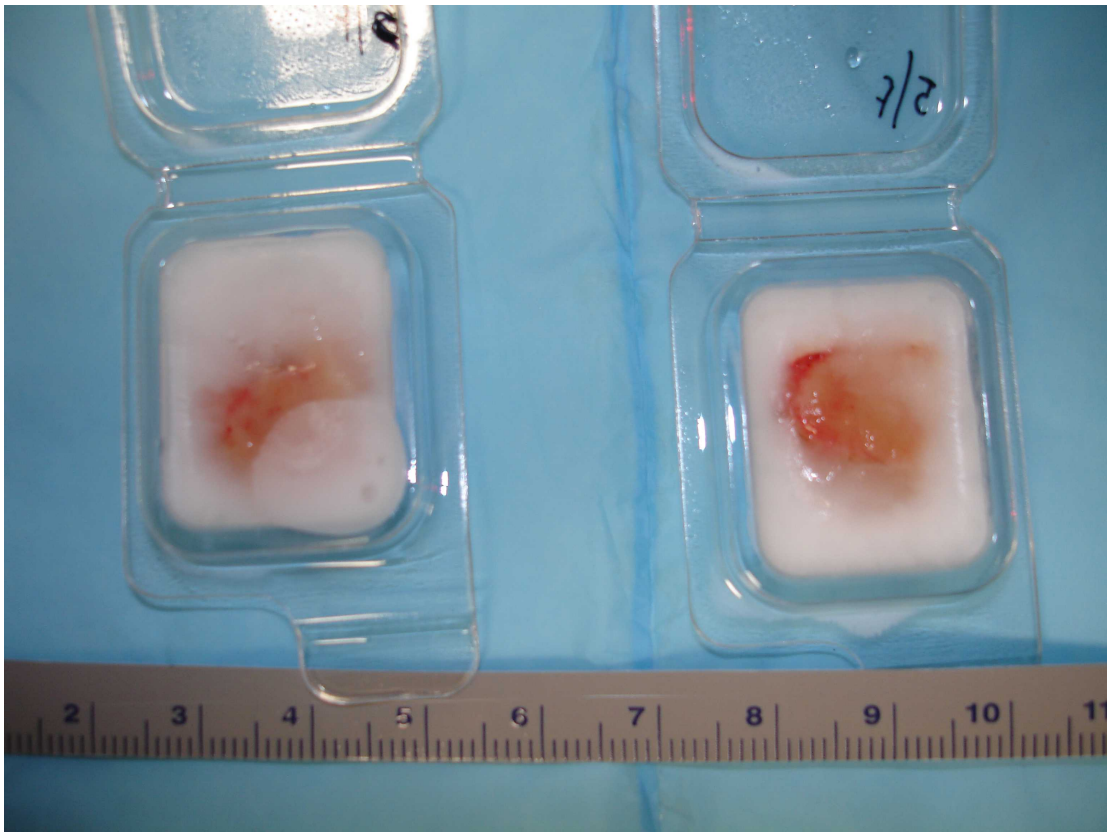

Figure 3. The tissue on the left is properly covered with OCT but the tissue on the right is NOT properly covered with OCT. The tissue must be completely covered with OCT to ensure specimen integrity. **Note that this should be performed on dry ice. This picture was taken on the bench top to avoid taking a picture where the tissue is not visible due to the vapor coming off the dry ice.

6 On occasion, we may be requested to use the Liquid nitrogen method (holding the clamshell with forceps on top of the liquid nitrogen), and investigators' should be advised that this procedure might cause samples to be incompletely processed, as a layer of gaseous nitrogen can form at the sample surface reducing the heat transfer considerably. CHTN VUMC will only procure using this method when requested.

7 After the OCT has hardened, place the clamshell in the freezer and log the location in the donor IT system.

8 Properly dispose of all biohazardous waste and other waste.

Troubleshooting

9 The following troubleshooting guide can provide you with some common problems that have been seen:

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FAULT	CAUSE
Cracks appear in the frozen tissue	Freezing too rapidly Specimen is overly large Specimen contains large amounts of water
Specimen detaches from the microtome chux (stub)	Insufficient embedding medium
Tears in sections while microtoming	Embedding media (OCT) was frozen too quickly before tissue was added and additional OCT. This causes “sections” of OCT being frozen on each stage of OCT aliquoting. Embedding media (OCT) was aliquoted too quickly causes bubbles/air spaces in the OCT. When the histologist cuts the block, the air space causes sections to teat.
Sections skew to one side	Embedded tissue incorrectly oriented Embedding media added unevenly
Sections detach from the slide	Specimens contain overly fatty tissue, which are tissues that do not optimally embed

Table 1. This troubleshooting guide provides common faults and potential causes.

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