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Version 1

Freezing Fresh Tissue V.1

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Protocol status: Working

We use this protocol and it's working

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Abstract

Scope:

To describe the procedure for freezing fresh tissue procured from the Cooperative Human Tissue Network.

Expected Outcome:

Frozen tissue should have minimal structural damage from freezing.

Guidelines

Definitions:

1. Isopentane is 2-Methylbutane
2. CMC is carboxymethylcellulose

Materials

Reagents:

1. Dry Ice
2. Isopentane, Fisher O3551-4
3. Carboxymethylcellulose, Fisher C9481
4. Richard-Allan Scientific Neutral Buffered Formalin (10%), Fisher 22-050

Equipment:

1. Styrofoam box with lid or solvent compatible ice bucket
2. Ruler
3. Forceps
4. Tissue Cassettes
5. Aluminum Foil, Fisher 01-213-100
6. Aluminum Weigh Dishes, Fisher 08-732-103
7. Thermo Scientific Peel-A-Way Disposable Embedding Molds, 20mm x 40mm, Fisher 18-41

Reagent Preparation:

2.6% Carboxymethylcellulose:

Add 500mL water to 500mL bottle

Microwave for 2 minutes

Place bottle on heated stir plate $\sim 70^{\circ}$

Slowly add 13g to warmed water

Stir on low hotplate overnight, occasionally tightening lid and shaking bottle

Troubleshooting


Safety warnings



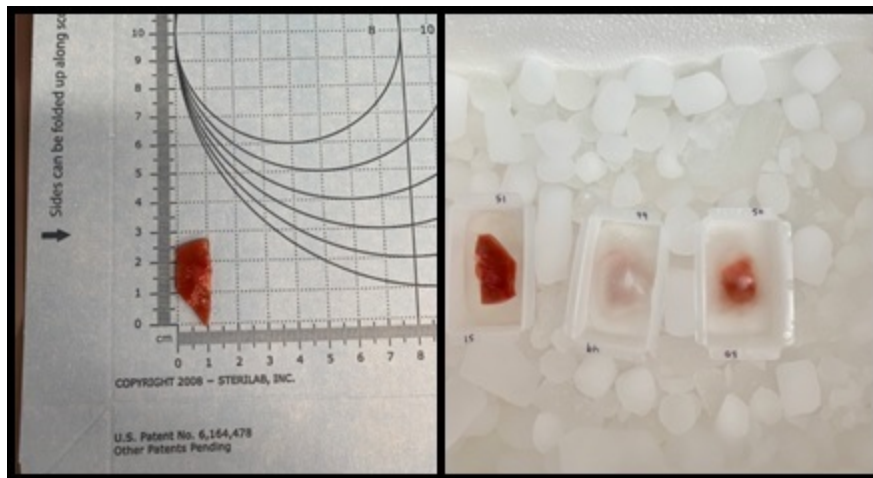
Health and Safety:

1. Safety glasses or goggles, proper gloves, and a lab coat required. The area should be adequately vented and a lab mat placed underneath all solutions and tissue.
2. Warning: Isopentane: Harmful by inhalation, in contact with skin and if swallowed. Keep container tightly closed and insure adequate ventilation. Flammable liquid and vapor.


Freezing and Documentation

- 1 Place a layer of dry ice in to box and add enough isopentane to make a slurry.
- 2 Label embedding molds and add a small amount of CMC to each one (barely cover the bottom).
- 3 Remove sample cup from ice and document on sample sheet the information on lid.
- 4 Remove sample with forceps. Measure and document the length, width and height. Document size and conditions (attachment)  Accession-Freezing Documentatio...
- 5 Place metal weigh boat on top of slurry and let cool. Add tissue and cover box with lid.
- 6 Allow tissue to completely freeze.
- 7 Place embedding mold with CMC on top of the slurry.
- 8 Check the mold in the slurry to see if it is starting to freeze. Add tissue when the CMC becomes only "tacky" on top. Orient the tissue so that the side that is to be cut is face down.
- 9 Slowly add more CMC on top of the tissue until it is covered. Keep the lid closed over the slurry as much as possible.

10



11 When completely frozen, wrap in foil and label sample number with sharpie.

12 Place all samples from one patient in a labeled box and place in the  -80 °C .

13 Place disposable equipment in the Biohazard trash. Wipe all forceps, rulers, bottles with appropriate cleansing wipes.

Formalin Fixed Tissue

14 If fixing in formalin, prepare 1 large jar of 10% formalin.

15 During step 4, carefully slice a small section of tissue (preferably having both cortex and medulla). Do this in a way that you create two "bookends."

16 Carefully place the piece of tissue into an embedding cassette.

17 Allow tissue to "fix" overnight.

18 Move tissue cassettes into 70% ethanol.



19 Tissue is then paraffin embedded by the pathology Core.