Cryo Sectioning of Undecalcified Bone

Although methods are well established for the rapid histological preparation of fresh frozen soft tissues, they have until recently remained unsuitable for the preservation of hard tissues. While the reliable technology of embedding hard tissue in plastic for sectioning preserves morphology well, its application for immunohistochemistry still remains suspect.

During the last 10 years, protocols for successful frozen sectioning of undecalcified bone have been developed and made routine. These protocols allow more rapid diagnosis of various bone disorders in the clinic and investigation of the histochemistry of bone in the research laboratory.

Freezing the Sample

The goal is to freeze your sample to at least -70° C. This temperature is well below the capabilities of the freezer bar in any commercial cryostat so other freezing means are needed.

Prepare your sample by first reducing its size to a maximum of 10 mm on a side. Smaller samples will be more uniformly and quickly frozen. Immerse the sample for 30 seconds in a 5 vol.% solution of polyvinyl alcohol in water (PVA). This PVA coating will help with adhesion of the sample to the cryostat chuck and helps prevent cracking of the bone during freezing.

A slurry of alcohol and dry ice will create a temperature below -70° C. Precool a bath of n-hexane (use a grade low in aromatic hydrocarbons) in this slurry. Isopentane is another popular cryogen for this application. Immerse your sample in the n-hexane and let stabilize for at least 30 seconds and not longer than about two minutes. From now on, only precooled tools should be used when handling the frozen specimen. Remove the tissue, absorb excess n-hexane with a cold filter paper, and store the frozen tissue in sealed dry tubes kept within the alcohol/dry ice slurry. Long term storage of your freezing apparatus and samples can be done in a low temperature freezer.

Preparing to Section in the Cryostat

Pour a bit of the 5 vol.% PVA solution on a microtome chuck and chill it on either the cryostat freezer bar or the alcohol/dry ice slurry. When a pool about the size of your specimen remains unfrozen, remove your sample from its dry storage tube and orient it in the gelling PVA. Once the PVA has finished freezing, let it stand for a time to insure that the PVA is completely frozen.

Cryostat sectioning of undecalcified bone is done with a tungsten carbide-tipped microtome knife. Use an unused area of the edge to collect your good sections and used areas of the edge for trimming, etc. The microtome knife must be much colder than normal cryostat sectioning temperature so that the energy generated when sectioning this hard material can be absorbed without raising the section temperature so much that damage from thawing occurs. By using a tungsten carbide knife with a tool steel base and packing the ends of the knife in dry ice, we can insure that the knife is at the proper temperature and that the cutting edge temperature changes minimally during sectioning.

Orient the sample chuck in the microtome arm so that the smallest edge of the blockface will strike the knife edge first. Double check all mounting screws and clamps to insure they are tight and secure.

Getting the temperatures of knife, sample and chamber correct independently and in relationship to each other is critical to obtaining good sections. The dry ice packing keeps the temperature of the knife at -70° C. The sample temperature will be the same as the chamber temperature unless it can be controlled to -60° C separately. The chamber temperature should be set to -30° C. The knife and chamber should be left at temperature for at least one hour to insure they have reached equilibrium.

Sectioning

You are ready to section once the sample has been clamped in the chuck, the knife has been clamped in the knife stage, the temperatures have been set and reached, and one hour has passed to allow thermal equilibrium. The microtome advance should be fully rewound and the

clearance angle set at 10° to 15° initially. Select a slow speed and set the sectioning window as appropriate for the blockface size. Trim by manually advancing the knife until a suitable block face is achieved. Clamp the knife stage in place and continue sectioning at 3 to 20 microns thick in automatic mode. Adjust section thickness, sectioning speed and clearance angle as needed to get nice looking sections.

Section Collection

The use of the anti-roll plate is appropriate for bone sectioning just like with soft tissue sectioning. Adjust the height, orientation and gap of the anti-roll plate when trimming so that it is in the right position during sectioning. Once you have a properly cut section, without curling or cracks, under the anti-roll plate, you are ready to collect it on a slide. Flip the anti-roll plate back and bring a room temperature slide up to and parallel to the section. Normally, the section will move by itself to the slide before you actually make contact with it. Remove the slide immediately from the cryostat and store in a dessicator.

Source:

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Citations:

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