Technical Excellence In Muscle Biopsy Preparation

Clint Lincoln A.S., HT(ASCP), IHC

More than a quarter of a century after the publication of Dubowitz and Brooke's classic *Muscle Biopsy: A Modern Approach in* 1973, there seems to be a lingering confusion regarding the proper technique for freezing muscle biopsies, and whether or not enzyme activity will be preserved. I have worked with muscle from humans, dogs, and rodents in my quarter of a century in histology, and would like to address the proper technical procedure to follow to insure high quality sections.

The first step is to acquire tissue immediately after excision, as any delay may alter the morphologic and enzymatic characteristics. If there is a slight delay between excision and freezing, keep the tissue covered with a gauze moistened (not soaked) in normal saline.

The next step is to actually perform the snap-freezing. This is an area where most mistakes occur and compromise the subsequent staining and interpretation. The sample should be immersed in isopentane (=2-methylbutane), which has been cooled in liquid nitrogen (LN_2). I fill a wide, 9" X 9" deep Dewar flask to about 4" depth with LN_2, and place a 200 mL stainless steel beaker filled with the isopentane in the Dewar flask. When the isopentane starts forming white pellets on the sides of the beaker, then the temperature is approximately -150° C and ready for snap-freezing.

At this point, one may elect to freeze the muscle "as is" in the isopentane for 10 seconds, or alternatively to position the sample on a cork base to which a layer of 10% gum tragacanth has been applied, and then freeze. The specimen is *not* immersed in the gum, but just placed in a 1 to 2 mm layer, and then the whole apparatus is frozen for 10 seconds. This time is suitable for specimens up to 10 mm long by 10 to 20 mm in diameter.

After freezing, the specimens may be placed in a suitable air-tight container for storage at -80°C. I use plastic scintillation vials and in each vial, I place a small piece of ice. The ice acts to keep the tissue from becoming freeze-dried over of time in the -80°C freezer. I have cut muscle that has been stored for from 3 to 5 years without artifact or loss of enzymatic activity.

To cut sections of muscle frozen without a cork base, one simply orients the tissue in a 1 to 2 mm thick layer of OCT (Optimal Cutting Temperature) compound on a specimen chuck. Samples mounted on a cork base are attached to the chuck by freezing the base to the chuck with a drop of water. The optimal cryostat temperature is around -20°C. *Note:* if one uses silane slides, and finds a propensity for the section to "fly" up to the glass, merely breathe on the back of the slide so your breath forms on it, and then pick up the section on the front side of the slide with ease. If this technique is followed, one should routinely acquire sections of the quality shown in Figure 1.

The most common cause of artifact in muscle preparation is slow freezing, and thus ice crystal formation. This translates into a "moth-eaten" appearance of muscle fibers and obscures the true nature of the pathology, as well as destroying evidence of "central core disease" and others. This is particularly important since some myopathies and neuropathies show pathologic "moth-eaten" fibers.

If one freezes tissue solely in LN_2, the warm tissue forms a vapor barrier around itself and slows the cooling, causing the artifact shown in Figure 2. If the sample is immersed in OCT and then frozen, even though in the correct freezing medium, the OCT creates a thermal barrier causing the larger ice crystal artifact shown in Figure 3. Some labs use a dry ice/isopentane medium (-70°C) for freezing. When using this coolant mixture to freeze muscle that is immersed in OCT, the greatest ice crystal artifact is produced, as shown in Figure 4.

It is best not to use such things as methanol or isopentyl alcohol, as these cause a fixation hardening on the surface of the muscle, and leave a greasy residue that inhibits quality cutting. Some labs wrap the muscle with a piece of membrane such as diaphragm or peritoneum. This too can cause a slow down of cooling and lead to ice crystal artifacts. I have had cases that were sent to me with these membrane wraps that had been frozen in isopentyl alcohol and still held quantities of the alcohol. This keeps the section from cutting properly, as isopentyl alcohol does not freeze at -20° C.

Proper care in the initial handling of muscle specimens is an exacting requirement, and if practiced conscientiously, will insure that the diagnosis and subsequent clinical treatment of patients will not be compromised. ■

Dubrowitz, V. and M.H. Brooke. *Muscle Biopsy: A Modern Approach.* 1973. W.B. Saunders, London.
Braund, K.G. and C.E. Lincoln. Histochemical Differentiation of Fiber Types in Neonatal Canine Skeletal Muscle. 1981. Amer. J. Vet. Research. 42(3):407-415.

The review of this article by Donna Mendrick, Ph.D is much appreciated

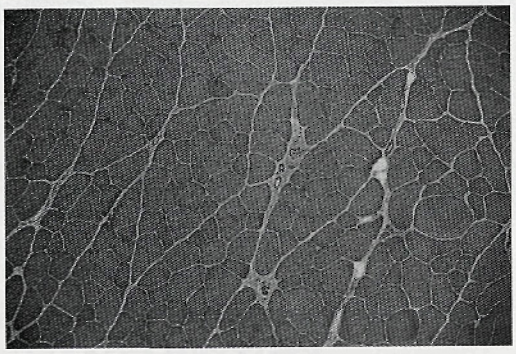


Figure 1: Frozen in isopentane, cooled in LN_2

Figure 2: Frozen in only LN_2

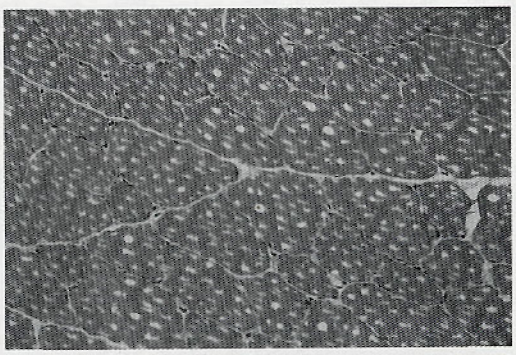


Figure 3: Immersed in OCT medium, then frozen in isopentaane cooled in LN_2

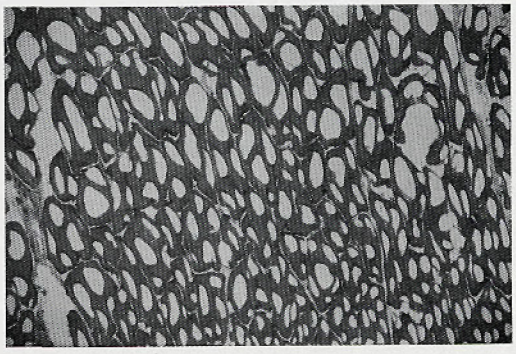


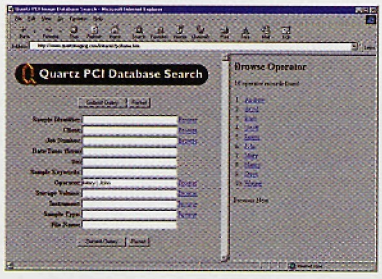
Figure 4: Immersed in OCT medium, then frozen in isopentane cooled in dry ice

Keeping a good image around the lab just got a lot easier. Quartz PCI.

With the Windows®-based Quartz PCI™ system, you can capture and manage high-resolution digital images from just about any image-producing device. And do so with a single—and familiar—family of software that lets you smoothly work with SEMs, STEMs, TEMs, light microscopes, video sources, TWAIN devices and CD SEMs. You end continuous learning of new software. Easily network diverse instruments. And conveniently enter all types of images into one database.

Further, after acquiring an image, PCI's processing and display capabilities let you do just about anything you want with it—from annotating, zooming, smoothing and sharpening it to making gamma adjustments and constructing anaglyph stereo images. And you're free to add your own custom functions as well.

Now, think about your database. PCI's scalable family of database solutions ensures your images—plus related documents, custom fields and tables—become part of a powerful database that provides data when, where, and in the form needed. OBDC compliance ensures compatibility with third-party tools like Microsoft® Access™ And PCI's Workgroup Database, for facilities with up to 25 users, is upgradable to our Enterprise Database which—based on either Oracle® or Microsoft® SQL Server™—supports thousands. With our Intranet Image Server, you can perform queries and retrieve images via standard Web browsers. And, for off-line storage, there's built-in support for removable media such as CD-ROMs or magneto-optical disks.



The PCI Intranet Image Server lets you use your Web browser to search for images.

So. If easily acquiring, analyzing, manipulating, annotating, transmitting, archiving and retrieving high-resolution images from various sources is in your future, you'll want Quartz PCI details in your hands. You can arrange that easily, too. Visit the Nissei Sangyo Web site for a demonstration, or let us hear from you by phone, FAX or E-mail.

NSA

Nissei Sangyo America, Ltd.
755 Ravendale Drive
Mountain View, CA 94043
(800) 227-8877
E-mail: sidsales@nissei.com
www.nissei.com

25 West Watkins Mill Road
Gaithersburg, MD 20878
(800) 638-4087

Circle Reader Inquiry #9