# Comments on "Technical Excellence in Muscle Biopsy Preparation" by Clint Lincoln (April, 1996)

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The Salk Institute, La Jolla, CA

I use any type of "tin" can, of various sizes, for my isopentane container, and they are free!

Parafilm can be placed between the cork and specimen to prevent it from sticking to the cork. This is helpful when re-embedding for cross-sections. Specimens can also pinned to the cork to maintain correct lengths.

I have frozen large muscle specimens, average size about 5 cm x 2 cm x 1 cm, but they must be left in isopentane for at least one minute or even longer. If the specimen is not completely frozen, it will have "moth-eaten" fiber artifacts in the center.

After freezing large specimens, they can be put in polypropylene centrifuge tubes cooled in liquid nitrogen.

I use plastic embedding molds to re-embed specimens for cross-sections:

1) Fill the mold with OCT and place it in a cryostat to cool (about 1 to 3 minutes). Before doing this, you may want to dissect the specimen with a cooled razor blade to obtain the best cross-section (this is all done in the cryostat chamber).

2) The specimen is put in the mold filled with cooled OCT before the OCT starts to freeze.

3) Be sure that the specimen is covered with OCT, and quickly immerse the mold in isopentane cooled with liquid nitrogen (same set-up as for freezing the original specimen). This may sound cumbersome, but this will eliminate ice artifacts, including on the edges, that occur if the specimen is put in warm OCT and/or frozen in the cryostat.

4) After the specimen is in the molds, it can be stored in small zipper bags.

Never reuse isopentane, since one never knows if there is any contamination, and it is not worth the cost if there is.

Comment by Clint Lincoln:

Ms. Kitabayashi's comments reflect the diversity of techniques that are used by technicians around the world. I'm sure that in her hands these techniques work, and work well. I applaud any one who finds an easier way of doing the work as long as quality is not sacrificed. I believe my article brought home the point that regardless of what manipulations one chooses to use, the bottom line for proper morphological presentation in muscle histochemistry is *proper initial handling.* Further, when an article such as mine stimulates techs to share their "secrets" through an open forum such as Microscopy Today, we all win!

Comment by Microscopy Today:

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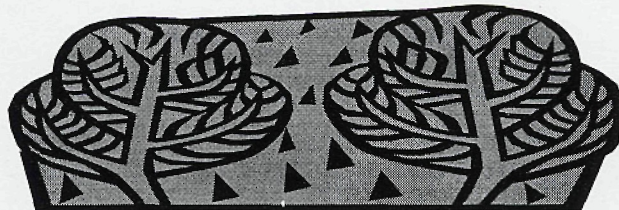

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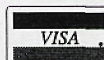

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