SECTION III

CURATION OF COLLECTIONS OF FROZEN TISSUES

CURATORIAL PROBLEMS UNIQUE TO FROZEN TISSUE COLLECTIONS

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In view of the fact that 95% of the collection managers surveyed maintain primarily frozen materials in their collections, and these collections are composed primarily of vertebrate tissues and blood (see Section IV), it is appropriate that we restrict our comments here to these kinds of collections. Managers of other kinds of collections (e.g., preserved cell lines and frozen semen collections) have, in the past, faced unique problems of their own; solutions to many of these problems have been found, largely because of widespread commercial interest in these collections.

Frozen tissue collections present not only the usual array of problems associated with the establishment and maintenance of systematic collections, but also several unique problems as well. Following are some general observations, comments, and recommendations, resulting from our efforts to establish organized and flexible frozen tissue curatorial programs at Texas Tech University (Baker) and Louisiana State University (Hafner). We are grateful for the knowledgeable advice provided us by James L. Patton, manager of the frozen tissue collection at the Museum of Vertebrate Zoology, University of California, Berkeley.

Cataloguing in the Field

In traditional systematic collections, specimens are collected in the field, assigned a collector's field number, brought back to the museum and catalogued. However, with regard to frozen tissues collected in the field, it is difficult to write a museum catalogue number on a frozen vial once it is returned to the laboratory because of the rapid accumulation of moisture on the outside of the vial. Therefore, we recommend establishment of a tissue collection catalogue (separate from the main collection catalogue) so that frozen tissues can be handled in a different manner than are traditional museum specimens. We use two, very different cataloguing procedures in our respective collections, both of which we feel are adequate. The Texas Tech collection maintains a series of hard-bound catalogues (see Figure III.1), each containing a unique sequence of catalogue numbers. Each collector or collecting party takes a bound catalogue into the field, and a tissue collection number is assigned to each specimen and written on the dry cryotube (using lead pencil, cryo-proof ink, or diamond-tipped pen) before the tube is placed in liquid nitrogen. Each tube is also labeled as to tissue type and genus (and species, if known) of the specimen from which the tissues were taken (see Chapter 7). On return to the museum, the tissue catalogue is immediately cross-referenced with the main collection catalogue.

TK 26076

Karyotyped by R. J. Baker Date 11 March 84
Collector Baker et al. Date 11 March 1984
Preparator KW Number 109
Museum TTU Number 40265

Freezer location Box AA, Ultracold II

Sex ♀ Species Onychomys leucogaster

State: TX County: Winkler

Specific locale: 17 mi N by Hwy 115 NE of Kermit, Rd to YT Ranch
S.B. Wright Jr.

Mitotic ✓ Meiotic No Tissue cultured NO

Liver ✓ Kidney ✓ Heart ✓ Blood NO Muscle NO

Other

Coordinates	2N	No. Biarm	No. Acro	Photo No.	Comments
A1 10.6 X 104.4	48	48	0	–	
A2 12.5 X 102.5	48	48	0	–	
A3 15.3 X 116.4	48	48	0	7859	Standard
C4 189 X 106.4	48	48		7860	G-bands
5 X					
6 X					
7 X					
8 X					
9 X					
10 X					

Remarks:
Not yeast stressed - 27 min in hypo

Figure III.1. Sample page of tissue collection catalogue used at Texas Tech University. Although this form is oriented toward karyological work, a modified version would be appropriate for most tissue collections.

In the mammal frozen tissue collection at Louisiana State University, each cryotube is labeled in the field with the collector's initials, the collector's unique field number, tissue type, and the scientific name of the tissue source. On return to the museum, the specimen is catalogued and assigned a unique frozen tissue collection number. This number, however, is not written on the cryotube, it merely serves as a convenient way to record the number of specimens in the collection. The critical marking on each tube is the collector's initials and field number (e.g., "MSH 1350"), which is used to retrieve specimens from the ultracold freezers. In the freezers, all specimens of a given taxon are stored together (see beyond); thus, the possibility of two different collectors having the same initials and field number for two specimens of the same taxon is extremely remote. This cataloguing procedure does not require the collector to take a bound tissue catalogue into the field and make entries into two catalogues (personal catalogue and tissue catalogue). Further, the collector who captures many more specimens than planned does not run the risk of using up all tissue numbers assigned to the particular catalogue volume. In both collections, we have found the practice of writing the scientific name of the animal on the cryotube to be extremely useful in subsequent curatorial and research work. In addition, we believe that it is wise to write the catalogue number (Texas Tech) or collector's number (LSU) twice on each tube to avoid the possibilty of the number rubbing off due to abrasion with nearby tubes in the liquid nitrogen tank.

Certain collection managers avoid the problem of relabeling wet tubes by transferring samples from field (plastic) cryotubes to cork-stoppered glass vials before placing them in the collection. We recommend against this procedure because cork stoppers are often not airtight, and transfer between vials usually requires thawing of the sample; both of these represent potential threats to the longterm stability of the tissues (see Chapter 6). We recommend the use of 2cc or 4cc plastic cryotubes with airtight gaskets (see Chapter 7) for both field collecting work and long term storage in the collection. Flat-bottom, plastic cryotubes are more expensive than glass vials, but the expense is minimal compared to the usual costs involved in collecting and storing frozen tissues. Cryotubes, unlike cork-stoppered vials, can be sterilized and reused once the sample is used or discarded. The survey revealed that most collections do, in fact, store tissues and blood in plastic cryotubes.

Storage in the Museum

Once samples have been catalogued in the field and returned to the museum, other problems in handling and storage are encountered. Thawing of samples should be avoided at all times during handling (see Chapter 6). Samples may be stored in an electrical freezer, on dry ice, or in liquid nitrogen. If freezers are used, they should be of the "ultracold" type so as to maintain samples at -70°to -90°C. (Most collections surveyed store their materials in ultracold freezers). These freezers may be either chest type or upright models; the former maintains more constant temperatures during use and is, therefore, less prone to mechanical failure over time. The upright models use less floor space, and freezer boxes are more easily retrieved from freezers of this design. Whichever model is selected, the freezer should be equipped with an alarm system that will sound in the event of electrical or mechanical failure. Most freezers are sold with a battery powered local alarm; at LSU we are installing a remote alarm system connected to the campus police station. With this system, our collection will be monitored around the clock, 365 days per year. Some form of backup storage system (other freezers, liquid nitrogen, or dry ice) should be readily avail-

able in the event of freezer failure (90% of U.S. collections are so protected). Ideally, liquid nitrogen is better for longterm storage because of its much colder temperature (-196°C). However, if large numbers of samples are stored in the collection, it may be difficult to retrieve specific samples from the liquid nitrogen tanks, and continual replenishment of evaporated liquid nitrogen may become costly. On a cost per sample basis, an ultracold freezer provides the most convenient and efficient method for longterm storage of large numbers of samples.

Another curatorial problem unique to frozen tissue collections involves the organization of samples within the freezer or liquid nitrogen tank for ease of access. In freezers, moisture-proof boxes labeled with numbers (or letters) can be used to sort samples by locality, or preferably, taxon. A listing of the holdings in each freezer, complete with box number, contents of the box, and location in the freezer is maintained and routinely updated as samples are moved, used, loaned or discarded. We recommend against storing the entire collection in numerical sequence because most access needs will be by taxon, and added freezer costs (due to loss of cold) are involved when searching for specimens scattered throughout the freezer(s). We recommend that freezers be opened as rarely as possible; one freezer should be set aside as a "working freezer" for storage of tissue samples that are currently being studied.

A small number of samples stored in liquid nitrogen tanks is relatively easy to organize for efficient retrieval. Each tank will hold six canisters, and each canister will hold approximatively 30 cryotubes. Each tank should be assigned a letter, and each canister a number. A file should be maintained indicating the contents of each canister. Again, it is possible to organize samples by locality, taxon, or any other category desired. If liquid nitrogen tanks are used, however, many tanks are needed if the volume of samples handled is high. Liquid nitrogen refrigerators are available that will efficiently organize up to 15,000 2cc cryotubes with easy access to any tube. However, a primary problem is initial cost, and such units have a high daily loss of nitrogen and hence require considerable yearly expenditures to maintain.

Acquisition Policies

The acquisition policies of a frozen tissue collection will vary with its freezer storage space, level of curatorial support, research interests of the curator (and other associated investigators), and ultimately, the collection's level of funding. Because maintenance of frozen tissues is relatively costly, we recommend that the curator pay very close attention to what is actually being catalogued into the collection. We recommend against cataloguing long series of samples from easily obtained organisms unless there is an immediate plan to utilize such samples in a research program. Unfortunately, the current level of support of frozen tissue collections prohibits the luxury of cataloguing and maintaining all samples received.

Managers of frozen tissue collections should seize every opportunity to acquire tissue samples of rare, unusual, and exotic species. When possible, samples of threatened or endangered species should be sought, but these (and all samples) should be acquired legally and used judiciously. Acquisition of tissues from certain kinds of organisms requires special permits (see Chapter 9). Collection files should contain copies of collecting permits issued to the original collector of the specimens, and if the material is imported into the United States, the files should contain copies of the required U.S. Department of Agriculture importation form 3-177 (see Chapter 9, Figure 9.1). All samples deposited in the collection should be documented as thoroughly as possible, much in the same manner as traditional museum specimens (Lee

et al., 1982). Except in rare cases, all samples should be represented by a traditional voucher specimen (skin, skull, or fluid specimen) deposited in an accredited systematic collection.

Deacquisition Policies

Unlike materials in conventional systematic collections, materials stored in frozen tissue collections are usually consumed as they are analyzed. Samples analyzed in museum laboratories and materials sent out on loan are rarely returned to the collection. The word "loan" therefore should be replaced with "gift" or "donation", when discussing transfer of frozen materials between collections and researchers. Because of this fact, much of the catalogued collection is only quasi-permanent, and a premium is placed on judicious dissemination of materials and efficient and flexible inventory procedures to keep track of deacquisitioned materials (discussed beyond).

We agree with the majority of survey respondents whose policy is to "loan" specimens only to carefully selected scientists (see Chapter 11). Selection should be based on the rarity of the specimen (and/or size of the sample) and the research direction of the potential recipient. Because samples are usually destroyed by the recipient, denials of requests for materials may be fairly common. It is the joint responsibility of the donor and recipient to be sure that transfer of the specimens is legal (i.e., the recipient may require special permits to handle the material; see Chapter 9). In every case, the naturalist who originally collected the tissues should be suitably acknowledged in any publication resulting from their use.

Shipments of frozen tissues are costly to package and transport. Our survey indicates that, at present, the donor (collection) pays tissue shipping costs 57% of the time and the recipient 43% of the time. In view of the fact that shipment is generally in one direction only (donor to recipient), we recommend that tissue shipment costs normally be paid by the recipient. *Investigators planning to obtain samples from frozen tissue collections for use in their research should provide for these costs in grant budgets.*

Computerized Inventory Systems

Ultracold storage space is very expensive to purchase and maintain, and it is important that materials be stored in a very space-efficient manner. It is therefore imperative that the access and inventory procedures for frozen tissue collections be extremely well organized. Ultracold freezers are very sensitive to even brief periods of temperature warm-up, and every second that a freezer door is open while a technician searches for a particular sample is energy consuming and could eventually contribute to freezer failure. In short, the curator (or technician) must know *exactly* where each sample is located *before* opening the freezer. Add to this the potential problem of wasted searches for samples that have been recently deacquisitioned (only 37% of the curators surveyed routinely update their catalogues), and the need for a flexible inventory system becomes even clearer.

Many of the unique curatorial problems posed by frozen tissue collections can be minimized through the use of a computerized inventory system. As a sample is "loaned" (= deacquisitioned), this deletion from the collection must be promptly recorded to avoid pointless and costly future searches for this sample. In addition, frozen tissue collection catalogues should be continually updated as specific tissues are homogenized or consumed, and as storage positions in the freezers are changed.

Important biochemical data, such as unique or marker alleles and the titer of antisera, should be recorded as they are obtained. A large number of data base management programs are currently available, most of which are adaptable for collection computerization.