

Frozen Biopsy Collection and Storage

Frozen Biopsy Samples

Russell Leek

Summary

This chapter describes some simple standard operating procedures for the regular collection of samples from surgical resections and their rapid preservation by freezing for long-term cryogenic storage.

Key Words: Biopsy collection; cryogenic preservation; inventory control.

1. Introduction

When collecting frozen human tissue of any type, the aim is to freeze the specimen in as short a period as possible following excision from the patient. In order to expedite this process, it is necessary to have an organizational structure in place to coordinate the people involved in chain linking the operating theater to the specimen. If biopsy collection is organized via anything apart from an *ad hoc* basis, then the regular cooperation of a host of health care professionals must be sought after and agreed on in advance. The cooperation of clinical staff is an absolute requirement for the smooth running of a regular biopsy collection service. Ideally, surgeons and pathologists should be consulted in advance, and a standard operating procedure should be agreed that does not interfere with the normal clinical routine (*see Note 1*).

Ideally, from the research laboratory side, it is desirable to have a dedicated technician available to collect specimens, as they come through the pathology reception at any time during reasonable working hours. If this person is allocated a hospital pager or mobile phone, then he or she is able to respond very quickly. Subsequent to tissue excision from the patient in the operating theater, the sequence of events should be as follows:

1. Biopsy is removed from patient.
2. Biopsy arrives at pathology laboratory.
3. Duty medical laboratory scientific officer calls for pathologist and biopsy collection research technician.
4. Pathologist dissects clinical samples from biopsy and supplies research technician with sample for cryogenic storage.
5. Research technician freezes sample in liquid nitrogen immediately (in the laboratory), and records details of the biopsy for inventory control.

Following this sequence, it should be possible to snap freeze a sample within 35 min of its excision. This is desirable, because experimentation has revealed that after 35 min, RNA degradation is rapid. This is especially important now, as many subsequent procedures involve the preparation of RNA from frozen material for the analysis of gene expression using systems such as microarrays.

1.1. Preparation

To support the short time frame necessary to freeze biopsies rapidly, a certain amount of preparation is valuable. In addition to gaining the cooperation of the professional staff involved and having an allocated “on-call” research technician, it is useful to have a biopsy collection kit that can be “grabbed on the run.” Such a kit should contain, as a minimum, the items listed in **Subheading 2**.

2. Materials

2.1. Biopsy Collection Kit Contents

1. Portable cryovessels containing liquid nitrogen.
2. Portable insulated box for dry ice.
3. Scalpel blades and handles (or disposable scalpels).
4. Plastic forceps.
5. Disposable gloves.
6. Cryovials to store tissue.
7. Indelible marker pen to write on cryovials (the ink must be able to withstand freezing to -180°C).
8. Pen.
9. Record book.
10. Protective eye shields.

3. Methods

As a priority, the pathologist will take whatever is deemed necessary for clinical diagnosis. Tissue for research may only ever be considered if there is sufficient material remaining following this primary objective. Material available for research should be divided between several cryotubes, so that back-ups are available in the future. This should be done at this point to reduce the number of future freeze–thaw cycles when samples are split. There are a number of

ways to freeze tissue samples, but the most effective and rapid way of snap freezing is to cut the biopsy into small pieces roughly 5 mm square, which are frozen directly by being held in liquid nitrogen, using a pair of long plastic forceps. The frozen piece is then placed immediately into a prelabeled tube (which has been precooled on dry ice) and stored temporarily on dry ice before being transported to the cryostorage unit (*see Note 2*).

When collecting biopsies, it is useful to consider their future use. If the sample is going to be used in its entirety, it is acceptable to freeze it directly in the tube (before closing the cap). If the sample is going to be split later, blocks should be frozen and placed in the tube separately, so they can be split without thawing. If the block is to be used for cryostat sectioning, it is worthwhile cutting the tissue into square blocks before freezing, so that it can be sectioned easily. It is also possible at this stage to mark the faces of the block with colored inks for orientation purposes.

It is also advisable to remember that it may be useful to have additional specimens of associated normal tissue as well as, for example, in the case of tumor specimens, samples of involved lymph nodes, or other metastases, where possible. These should be collected before the biopsy is preserved in fixative.

In order to preserve the patient's anonymity, it is useful to assign each new specimen an inventory number that can be tracked back to the patient. A single inventory number is a convenient way of labeling a tube without having too much information written on what is sometimes a very small label. The inventory number, pathology number, and hospital number may be written in the record book, which is kept in a secure place. This information may also be stored in a secure database as long as all of the conditions of the Data Protection Act (in the United Kingdom) are adhered to.

3.1. Long-Term Specimen Storage

The preferred method for long-term storage of frozen biopsies is in the vapor phase of liquid nitrogen at a temperature of less than -160°C . It is recommended that samples not be stored in the liquid phase in sealed tubes, as the tubes might explode when removed and the samples begin to thaw. Alternatively, storage in a -80°C freezer is acceptable and does not result in RNA degradation. A normal, -40°C freezer is unsuitable for storage of tissue specimens.

If important samples are to be stored on a long-term basis, it is advisable to keep duplicate samples in separate storage facilities, preferably in separate buildings.

3.2. Inventory Control

When embarking on a tissue collection project, some thought should be given to an inventory control system. This is an absolute necessity if the goal is to collect a tissue bank over a long period. New specimens in an inventory

should be assigned a coordinate in the storage bank. The nature of this coordinate depends on the storage system used, but would usually follow a sequential alphanumeric format. For example, in a circular liquid nitrogen vessel containing six triangular stacks of draws, a specimen in stack 4 in the fifth drawer down at position 27 could be assigned a coordinate of 4-5-27. This coordinate may then be entered against the specimen's inventory number in the catalog, so that it can be retrieved rapidly in the future. Small catalogs may be kept as written records, but for larger catalogs, it is advantageous to keep this information on a computerized database (such as Microsoft Access [Windows] or FileMaker Pro [Apple Macintosh]). When samples are recorded on a relational database, it is much easier to link them to other information about the biopsy, patient, and data generated from experimental work on the sample.

4. Notes

1. It may also be necessary to have the informed consent of the patient concerned that tissue be stored for research purposes.
2. It is inadvisable to place cryovials directly into the liquid phase of liquid nitrogen with the cap on, because they can explode when they are removed. Cryovials can only be stored in the liquid phase if they are first wrapped in cryoflex tubing.



<http://www.springer.com/978-1-58829-191-2>

Breast Cancer Research Protocols

Brooks, S.A.; Harris, A. (Eds.)

2006, XIV, 518 p., Hardcover

ISBN: 978-1-58829-191-2

A product of Humana Press