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🌐 NCI Biospecimen Evidence-Based Practices (BEBP) - Snap-freezing of Post-Surgical Tissue Biospecimens

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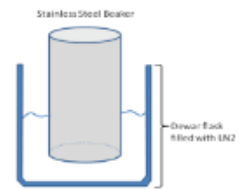
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Figure 7.1. Experimental set-up for snap-freezing in LN₂ vapor



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Protocol status: Working

This document contains guidance that is intended to facilitate the development of evidence-based standard operating procedures.

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Disclaimer

This document contains guidance that is intended to facilitate the development of evidence-based standard operating procedures.

Abstract

This evidence-based best practice document is applicable to all human tissues that are to be preserved by snap-freezing. Biospecimens preserved under these procedural guidelines are suitable for downstream analysis of DNA, RNA, protein, and morphology endpoints. Additional analytical endpoints, including but not limited to cell viability, cell sorting, drug sensitivity testing, or use as donor specimens for xenografts or primary tissue culture, do not fall within the scope of this document.

Attachments



[NCI_BEBP_Snap-freezi...](#)

1.1MB

Guidelines

9.1.1. Universal Precautions (CDC, 1987):

<http://www.cdc.gov/mmwr/preview/mmwrhtml/00000039.htm>; Tools for Protecting Healthcare Personnel, CDC: <http://www.cdc.gov/HAI/prevent/ppe.html>.

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Materials

5.1. Plastic-backed absorbent bench paper.

5.2. New disposable dissecting equipment for each organ.

5.3. Liquid Nitrogen (LN2).

5.4. Dewar flask.

5.5. Cryogenic specimen storage container (cryovial, cryostraw, cryosette[®], cryomold, or equivalent storage container designed for temperatures at or below -190°C), LN2 storage container or, in the event of immediate shipment, LN2 dry shipper.

5.6. Should LN2 be unavailable, alternative freezing media may include: isopentane pre-cooled with LN2; isopentane cooled with dry ice; dry ice alone; -80°C freezer. When utilizing dry ice or -80°C for freezing and storing at -80°C, suitable cryogenic specimen storage containers designed for temperatures at or below -80°C will be acceptable and shipment may be performed on dry ice.

Safety warnings

 Universal Precautions (CDC-1978) are used for all phases of organ/tissue dissection and handling (See Reference 9.1.1).

Ethics statement

Protocols developed using this Biospecimen Evidence-Based Practice may require approval by the user's institutional review board (IRB) or an equivalent ethics committee prior to implementation.

Before start

The purpose of this document is to provide evidence-based guidance for the proper snap-freezing of human tissue biospecimens. This guidance is intended to support the development and execution of evidence-based Standard Operating Procedures (SOPs) for human biospecimen collection, processing, and storage operations.

Recording of biospecimen preacquisition data

- 1 Whenever possible, extensive data should be recorded relating to preacquisition conditions that may affect the integrity of the biospecimen. Such data may include patient information (including age, gender, diagnosis, and treatment) as well as details relating to surgery and biospecimen acquisition (including the use of anesthesia, warm ischemia time, and surgical procedure and duration).

Preparation of freezing containers and bench space

- 2 Pre-labeled cryogenic specimen storage containers for each organ being dissected should be identified and arranged before the organ is available for dissection.
- 3 Specimen containers should be appropriately labeled and organized, and tissues of different anatomic sites as well as tumor and normal tissues should be segregated to the extent possible.
- 4 Clean disposable scalpels and forceps should be used when cutting different tissue types of the same patient and specimens from different patients. Contact with absorbent materials that may contaminate dissected research tissues or where capillary action may draw fluid from tissue samples should be avoided.

Post-collection storage of tissue specimens on wet ice

- 5 Specimens may be placed in a sterile closed container on  On ice until dissection (See Section 8.1 in the Attached PDF for Literature Evidence).

Minimizing cold ischemia time

- 6 Dissection should be accomplished soon after the specimen is released by the supervising physician. Cold ischemia time should be minimized as much as possible, optimally less than 20 min but no more than  01:00:00 (See Section 8.2 in the Attached PDF for Literature Evidence). Cold ischemia time should be documented for every module or segment and for each subsequent aliquot.
- 7 For tissue specimens collected postmortem, postmortem interval (PMI) should be minimized as much as possible, optimally less than 2 hours, but no more than 6 h (See Section 8.3 in the Attached PDF for Literature Evidence). This time should be documented for every patient and attached to the module and its aliquots.

Dissection notes

- 8 Dissection should be performed one organ at a time. Final aliquots should be no thicker than ± 0.4 cm and placed into the proper cryogenic specimen storage containers. If morphological analysis is anticipated, then specimens can be surrounded by Optimal Cutting Temperature (OCT) Compound prior to freezing; however, the use of OCT Compound is not optimal for some specific molecular analysis methods (See Section 8.4 in the Attached PDF for Literature Evidence).

Freezing of tissues

- 9 Optimally, the tightly sealed cryogenic specimen storage container should be frozen in liquid nitrogen (LN2) vapor. This can be achieved by suspending a stainless steel beaker inside a bench top Dewar flask pre-filled with LN2 (See Figure 7.1 in the Attached PDF). The specimen storage container should then be placed inside the steel beaker for  00:02:00 or less depending on the size of the specimen (See Section 8.5 in the Attached PDF for Literature Evidence). Common alternatives to freezing in LN2 vapor may include freezing by immersion in LN2 or immersion in isopentane precooled to  -80 °C or below (See Section 8.6 in the Attached PDF for Literature Evidence).
- 10 If LN2 is unavailable at the physical site where specimens are collected and preserved, alternative freezing methods may used, and include immersion in isopentane pre-cooled with dry ice, placement on dry ice, or placement in a  -80 °C freezer. Freezing specimens directly on dry ice should be avoided if they are to be used for morphological analysis (See Section 8.7 in the Attached PDF for Literature Evidence).

Transfer and storage of frozen biospecimens

- 11 After freezing, the cryogenic specimen storage container should be transferred for storage in an LN2 vapor freezer. Should LN2 be unavailable, specimen storage containers may be stored at  -70 °C or colder (See Section 8.8 in the Attached PDF for Literature Evidence).
- 12 Alternatively, the frozen specimens may be placed directly into an LN2 dry shipper for immediate transport (See Section 8.8 in the Attached PDF for Literature Evidence). Specimen containers frozen in LN2 and destined for storage in LN2 should be held in LN2 vapor before and during transfer to repository/long-term storage. Should LN2 be unavailable, specimen storage containers may be shipped on dry ice.



Specimen containers destined for storage at  -80 °C should be held on dry ice before and during transport.

Protocol references

References considered during the development of this NCI BEBP document are listed below (also See Section 9.2 in the Attached PDF) and include hyperlinks to the PubMed abstract and NCI Biospecimen Research Database curation where applicable. References are cited within the Summaries of Literature Evidence (See Section 7.0) in the Attached PDF.

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