Snap Freezing of Tissue

Category:	**Materials Handling and Documentation**		
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1.0 PURPOSE

The purpose of this SOP is to outline the standardized procedures for the snap freezing of tissue samples at Nourah's Tissue Biobank. Snap freezing is a critical process used to preserve the integrity of tissue samples for future research and analysis.

2.0 SCOPE

This SOP applies to all personnel involved in the snap freezing of tissue samples within Nourah's Tissue Biobank. It covers the steps required to properly freeze tissue samples to preserve their molecular and cellular integrity, including documentation using the LabVantage LIMS system.

3.0 ROLES AND RESPONSIBILITIES

This SOP applies to all personnel of Nourah's Tissue Biobank members

Biobank Personnel	Responsibility
Pathologist/Pathologist Assistant	Responsible for selecting and preparing tissue samples for snap freezing, ensuring they meet the necessary criteria.
Laboratory Technicians	Responsible for performing the snap freezing procedure according to this SOP, ensuring the proper handling, labeling, and documentation of frozen tissue samples.
Biobank Manager	Responsible for overseeing the snap freezing process, ensuring compliance with this SOP, and addressing any issues that arise.
Quality Assurance (QA) Officer	Responsible for auditing the snap freezing process, ensuring adherence to protocols and regulatory requirements.

4.0 MATERIALS, EQUIPMENT, AND FORMS

Listing of the materials, equipment, and forms being used to achieve the goals of the SOP, this list will mainly contain necessary materials and, or recommendations that may be substituted by alternative or equivalent materials more suitable at the time of testing.

Material to be used	Site
Liquid nitrogen or a dry ice-isopentane bath	
Isopentane (when using dry ice for freezing)	
Cryovials or other suitable containers for tissue storage	
Labels and markers	
Forceps and scalpel	
Insulated gloves and face shield (PPE)	
Insulated transport containers (e.g., dry shippers)	
Biohazard waste containers	

5.0 POTENTIAL HAZARDS

In this part of the SOP, we explain the potential hazards from chemicals and methodologies used in this procedure. It will also contain information on how to handle these chemicals and the level of biosafety

Material	Safety and handling

6.0 PROCEDURES

This section outlines the steps involved in preparing, freezing, and documenting tissue samples through snap freezing at Nourah's Tissue Biobank. These procedures ensure the rapid preservation of tissue samples, maintaining their molecular integrity for future analysis.

6.1 PRE-FREEZING PREPARATION

1. The pathologist or pathologist assistant selects tissue samples for snap freezing based on research or diagnostic needs.
2. The selected tissue should be appropriately sized for freezing, generally no thicker than 0.5 cm to ensure rapid and uniform freezing.
3. Set up the freezing apparatus, either by preparing a liquid nitrogen container or by creating a dry ice-isopentane bath.
4. Ensure that all cryovials or storage containers are pre-labeled with the tissue type, patient identifier, date, and any other relevant information.
5. Wear appropriate PPE, including insulated gloves and a face shield, to protect against the extreme cold during the snap freezing process.
6. Ensure that the work area is well-ventilated, especially when using isopentane.

6.2 SNAP FREEZING PROCEDURE

1. Freezing Using Liquid Nitrogen
    a. Transfer the tissue sample with sterilized forceps to a pre-labeled cryovial
    b. Hold the tissue sample and quickly immerse it into the liquid nitrogen until fully frozen (typically within seconds).

c. Immediately place the vial in the liquid nitrogen for storage.

2. Freezing Using Isopentane bath
    a. Prepare the isopentane bath by adding isopentane to a sterilized metalic container
    b. Allow the isopentane to cool to the freezing point by placing the metallic container in liquid nitrogen.
3. Hold the tissue sample with sterilized forceps and immerse it in the isopentane until fully frozen.
4. Transfer the frozen tissue sample to a pre-labeled cryovial and store it in liquid nitrogen or another appropriate ultra-low temperature environment.

6.3 LABELING AND DOCUMENTATION

1. Ensure all cryovials are labeled with the necessary information, including patient identifier, tissue type, date, and freezing method used.
2. Verify that the labeling is legible and securely attached to the cryovials.
3. Enter the details of the snap frozen tissue samples into the LabVantage LIMS system immediately after freezing.
4. Document the method of freezing, time, and any observations regarding the condition of the tissue before and after freezing.

6.4 TRANSPORTATION

1. Store the cryovials containing the snap frozen tissue samples in liquid nitrogen or an ultra-low temperature freezer (-80°C or colder) as soon as possible after freezing.
2. Ensure that the storage location and conditions are updated in the LabVantage LIMS system for accurate tracking.
3. Snap frozen tissue samples need to be transported, place the cryovials in an insulated transport container with liquid nitrogen or dry ice, depending on the destination.
4. Record the transport details in the LIMS, ensuring traceability during transportation.system.

6.5 INCIDENT MANAGEMENT

1. In case of any deviations from the SOP (e.g., temperature excursions, delays), document the incident and notify the Biobank Manager immediately.
2. Implement corrective actions as necessary to mitigate any impact on sample integrity.
3. Complete an incident report detailing the deviation, corrective actions taken, and any follow-up measures required.
4. Submit the report to the QA Officer for review and documentation.

7.0 REFERENCES

1. CTRnet SOPs "08.03.003 Snap Freezing of Tissue"
2. Declaration of Helsinki.
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3. Human Tissue and Biological Samples for use in Research. Operational and Ethical Guidelines. Medical Research Council Ethics
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4. Best Practices for Repositories I. Collection, Storage and Retrieval of Human Biological Materials for Research. International Society for Biological and Environmental Repositories (ISBER).
   http://www.isber.org/Search/search.asp?zoom_query=best+practices+for+repositories
5. National Bioethics Advisory Commission: Research involving human biological materials: Ethical issues and policy guidance, Vol. I: Report and recommendations of the National Bioethics Advisory Committee. August 1999.
   http://bioethics.georgetown.edu/nbac/hbm.pdf
6. US National Biospecimen Network Blueprint
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8.0 REVISION HISTORY

SOP No.	Date Revised	Author	Summary

9.0 APPENDICES