National
Liver
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Biobank

Standard Operating Procedure

DOCUMENT NAME: MATERIALS HANDLING AND DOCUMENTATION

DOCUMENT NO. :ILBS#NLDB:H

MATERIALS HANDLING AND DOCUMENTATION

Labeling and Tracking Materials

Document Name : Labeling and Tracking Materials
Document No. : ILBS#NLDB:H.1
Version No. : 1.0
Effective Date : 01/01/2025

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MATERIALS HANDLING AND DOCUMENTATION

Labeling and Tracking Materials

Number	Effective date	Pages	Author	Authorized by
ILBS#NLDB:H.1	01/01/2025	7	Mr. Satish Kumar	Dr.Chhagan Bihari
Version	**Review period**	**No. of copies**	**Approved by**	**Date**
1.0	2yrs	3	Dr. Chhagan Bihari	30/12/2024

Location	Subject
Biobank Reception area Almira	Labeling and Tracking Materials
Function	**Distribution**
To give information about Labeling and Tracking of Materials.	➢ HOD ➢ Biobank Reception area ➢ Master files

SCOPE AND APPLICATION:

This SOP describes how samples are labelled and tracked.

RESPONSIBILITY:

- Nurses obtain patient consent.
- Phlebotomists collect blood.
- Biobank staff handle transport and processing.
- Biobank HOD oversees operations and quality assurance.

MATERIALS HANDLING AND DOCUMENTATION

Labeling and Tracking Materials

ABBREVIATIONS

Ca	Cancer
OT	Operation Theatre
CT	Chemotherapy
HT	Hormone Therapy
Post NACT	Post-Neoadjuvanvt Chemotherapy
POST RT	Post Radiotherapy
BC	Buffy Coat
PP	Plasma
NT	Normal Tissue
TT	Tumour Tissue
UIN	Unique Identification Number
Rpm	Revolution Per Minute
DNA	Deoxyribo Nucleic Acid
RNA	Ribo Nucleic Acid
MRD	Medical Record Department
EDTA	Ethylenediaminetetra acetic Acid
BRC	Bio samples Release Committee
PAC	Pre Anesthetic Check up
QC	Quality Control
bp	base pairs
DMSO	Dimethyl Sulfoxide
FCS	Fetal Calf Serum
PBMC	Peripheral Blood Mononuclear Cells
RT	Room Temperature (18°-25°C)
EDTA	Ethylene Diamine tetra acetic acid
ACD	Acid citrate dextrose

MATERIALS HANDLING AND DOCUMENTATION

Labeling and Tracking Materials

1.0 PURPOSE

The purpose is to outline general procedures that can be used by NLDB to ensure that labelling and tracking are maintained with essential standards to prevent loss of samples due to inadequate identifying information.

2.0 SCOPE

This SOP describes how samples are labelled and tracked.

3.0 ROLES AND RESPONSIBILITIES

This SOP applies to all personnel from NLDB that are responsible for obtaining, processing storing and tracking human biological samples in the biobank. Applicable staff may include the following roles:

Nurse: Obtain Patient Consent

Phlebotomist/ Venipuncture nurse: Draw Blood from patient and read and understand product inserts.

Biobank Staff: Transport and Process blood.

PI or biobank manager: Responsible for Operation and Quality Assurance at the biobank

4.0 MATERIALS, EQUIPMENT AND FORMS

- Appropriate Labels such as Cryogenic Thermal Transfer-Tags
- Computerized Inventory system
- Label printer & Scanner

5.0 PROCEDURES

This procedure is intended to ensure that samples obtained from consented participants are appropriately identified and tracked to eliminate the risks of sample misidentification and loss.

5.1 Labelling of Samples

5.1.1 Label all level of receptacles containing human biological samples or products, from the smallest unit (cryovial, histological slide, or filter) to the large storage units.

5.1.2 Make sure that each label used adheres tightly to the receptacle under all projected storage conditions. Do not use labels that will come off in liquid nitrogen or under specific conditions of heat or cold used for processing or storage.

5.1.3 Printing on the labels should be resistant to all common laboratory solvents and water (e.g., use a cryomarker, cold-resistant label, waterproof/solvent-proof pen, thermal-transfer printer).

5.1.4 Test adherence of labels to containers as well as different types of marking ink under different storage conditions before implementing the labelling method for routine use.

5.1.5 Only include information on the label that is compliant with applicable privacy legislation. Do not include patient identifying information. Identifying information such as name, date of birth, health insurance number, etc. must not be on the label.

5.1.6 Information should be specific enough so that the encoded information can be associated with the sample in the database.

5.1.7 Only include static information.

5.1.8 Consider labelling by computer and not by hand, as this will eliminate problems that arise due to variations in handwriting and misreading of labels.

5.1.9 Bar coded labelling system utilizing a linear (one dimensional) bar code that includes human readable identification of contents.

5.2 Tracking and Inventory System

A tracking and inventory system should be in place to ensure that a sample can be located at any time during collection, processing, storage, and distribution. The system should be capable of linking the sample to associated patient consent, clinical and research information. It should also be designed to ensure that the sample environment is kept as stable as possible during processing, storage, sorting and shipping.

5.2.1 Assign a unique identifier such as a tracking number or bar code to each sample at the time of collection.

5.2.2 Link the same identifier to all associated clinical and scientific data for the sample.

5.2.3 Update the inventory or tracking system to reject any movement or change in the sample or data within or outside the biobank.

5.2.4 Ensure that the inventory and tracking system is capable of generating a full audit trail of changes made to the database or system,

5.2.5 Control access to the computerized inventory very tightly.

5.2.6 Generate a unique identifier (address) for each freezer, refrigerator, or storage cabinet. Establish numbering for shelves, racks, boxes as well as each location within the storage receptacle.

5.2.7 Use the inventory system to track sample type, date of collection, volume and size of aliquots, history of sample movement, method and time of sample processing, shipment and thaws and deviations from regular storage conditions if relevant.

6.0 APPLICABLE REFERENCES, REGULATIONS AND GUIDELINES

- 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
- US National Biospecimen Network Blueprinthttp://biospecimens.cancer.gov/resources/publications/reports/nbn.asp
- Human Tissue and Biological Samples for use in Research. Operational and Ethical Guidelines. Medical Research Council Ethicshttp://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC002420

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Standard Operating Procedure

DOCUMENT NAME: MATERIALS HANDLING AND DOCUMENTATION

DOCUMENT NO. : ILBS#NLDB:H

Document Name	**:**	**Bio hazardous Material Waste Management**
Document No.	**:**	**ILBS#NLDB:H.2**
Version No.	**:**	**1.0**
Effective Date	**:**	**01/01/2025**

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MATERIALS HANDLING AND DOCUMENTATION

Bio hazardous Material Waste Management

Number	Effective date	Pages	Author	Authorized by
ILBS#NLDB:H.2	01/01/2025	6	Mr. Satish Kumar	Dr.Chhagan Bihari
Version	**Review period**	**No. of copies**	**Approved by**	**Date**
1.0	2yrs	3	Dr. Chhagan Bihari	30/12/2024

Location	Subject
Biobank Reception area Almira	Bio hazardous Material Waste Management
Function	**Distribution**
This SOP gives information about the safe handling and disposal of biohazardous waste in compliance with biosafety and biomedical waste management requirements.	➢ HOD ➢ Biobank Reception area ➢ Master files

SCOPE AND APPLICATION:

This SOP describes the procedure for safe handling and disposal of biohazardous waste in compliance with biosafety and regulatory requirements to minimize contamination and exposure risks to personnel and the environment.

RESPONSIBILITY:

This SOP applies to all authorized NLDB biobank personnel involved in collection, processing, storage, tracking, and disposal of human biological samples, including phlebotomists, technicians, and pathologists, as per assigned responsibilities.

ABBREVIATIONS

Ca	Cancer
OT	Operation Theatre
CT	Chemotherapy
HT	Hormone Therapy
Post NACT	Post-Neoadjuvanvt Chemotherapy
POST RT	Post Radiotherapy
BC	Buffy Coat
PP	Plasma
NT	Normal Tissue
TT	Tumour Tissue
UIN	Unique Identification Number
Rpm	Revolution Per Minute
DNA	Deoxyribo Nucleic Acid
RNA	Ribo Nucleic Acid
MRD	Medical Record Department
EDTA	Ethylenediaminetetra acetic Acid
BRC	Bio samples Release Committee
PAC	Pre Anesthetic Check up
QC	Quality Control
bp	base pairs
DMSO	Dimethyl Sulfoxide
FCS	Fetal Calf Serum
PBMC	Peripheral Blood Mononuclear Cells
RT	Room Temperature (18°-25°C)
EDTA	Ethylene Diamine tetra acetic acid
ACD	Acid citrate dextrose

1.0 PURPOSE

All Human Biological Materials (HBMs) whether fixed, lyophilized, fresh, frozen or paraffin embedded should be considered biohazardous. The degree of processing may reduce the risk from infective agents. However, certain agents may still be infective even when fixed or processed. All human specimens, independent of their state, should be treated with universal precautions. They should be handled as if infected with agents that may be pathogenic to humans.

2.0 SCOPE

This SOP outlines processes that must be followed in order to dispose of biohazardous waste in a manner compliant to bio safety regulations and ensuring that the following risks are minimized:

- Contamination of public waste sites with biohazardous materials.
- Exposure of biobank and waste management personnel to infectious agents.

3.0 ROLES AND RESPONSIBILITIES

This SOP applies to all personnel from NLDB biobanks that are responsible for obtaining, processing storing and tracking human biological samples in the NLDB biobank. Applicable staff may include the following roles:

Phlebotomist: Draw Blood from patient and read and understand product inserts

Technician: Collect and process biological material

Pathologist: Collect, Process and assess biological material

4.0 MATERIALS, EQUIPMENT AND FORMS

- Autoclave
- Waste disposal bags (appropriately labeled)
- Biohazardous sharps disposal containers
- Bleach or chemical disinfectant

5.0 PROCEDURES

Biobank must follow procedures regarding the disposal of biohazardous waste that minimized the risk it poses to the environment and to biobank personnel.

Procedures should ensure adherence to Indian, provincial and institutional guidelines. The biobank must ensure the use of appropriate waste management techniques, containment levels, and training of personnel.

5.1 Disposal – Human anatomical waste

5.1.1 Place all human anatomical waste and materials that have come into contact with such waste into a bag clearly labelled with the universal biohazard symbol.

5.1.2 Biohazardous waste must be decontaminated before disposal to a landfill site.

5.1.3 Decontaminate by heat sterilization (autoclaving) and take to the institutional designated area for pick-up and disposal.

5.1.4 Biohazardous waste that has not been decontaminated can be picked-up by an established waste disposal company for disposal. This may require that the biobank obtain a special ministerial permit granting approval for generation and disposal of waste by this procedure.

5.2 Disposal – Biohazardous Liquids (Human Blood and Body Fluids Waste)

5.2.1 Dispose of blood and liquid biohazardous waste generated during sample processing by pouring the waste into a leak proof container containing freshly prepared 10 % chlorine bleach solution or other suitable chemical disinfectant. If possible perform this task in a fume hood.

5.2.2 After 30 minutes or a suitable time-interval ensuring decontamination, the solution may be discarded down the drain if permitted by local regulations.

5.2.3 Avoid the creation of aerosols or spills during this process.

5.3 Disposal – Sharps Waste

5.3.1 Recapping of needles is not recommended.

5.3.2 Dispose of all sharps waste into a readily available, approved puncture resistant container labelled with the biohazard symbol.

5.3.3 Sharps containers must be decontaminated (preferably by incineration or autoclaving) and disposed of in accordance with institutional, national and provincial guidelines.

5.4 Disposal of Waste for Non-Bankable Samples

- In the case of inadequate or revoked consent, identify all samples associated with the participant.
- Before disposal confirm the identity of the samples.

5.4.1 Destruction of Frozen Tissue

a. Retrieve samples from storage unit.
b. Leave samples in storage vials or cryomolds.
c. Place sample in the autoclavable biohazard bag.
d. Ensure that bag containing waste is incinerated or disposed of by a company with a license to do so.
e. Record that the sample has been discarded so as to update inventory systems.

5.4.2 Destruction of Blood Samples

a. Retrieve blood samples from storage unit.
b. Dispose of tubes in the biohazardous waste bag for incineration or adequate disposal as per institutional procedure.
c. Record that the sample has been discarded so as to update inventory systems.

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DOCUMENT NAME: MATERIALS HANDLING AND DOCUMENTATION

DOCUMENT NO. :ILBS#NLDB:H

Document Name : Inventory verification
Document No. : ILBS#NLDB:H.3
Version No. : 1.0
Effective Date : 01/01/2025

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MATERIALS HANDLING AND DOCUMENTATION

Inventory verification

Number	Effective date	Pages	Author	Authorized by
ILBS#NLDB:H.3	01/01/2025	6	Mr. Satish Kumar	Dr.Chhagan Bihari
Version	**Review period**	**No. of copies**	**Approved by**	**Date**
1.0	2yrs	3	Dr. Chhagan Bihari	30/12/2024

Location	Subject
Biobank Reception area Almira	Inventory verification
Function	**Distribution**
To give information about Inventory verification.	➢ HOD ➢ Biobank Reception area ➢ Master files

SCOPE AND APPLICATION:

This SOP describes the procedure for inventory verification to ensure correct recording and validation of storage locations in the computerized inventory system.

RESPONSIBILITY:

Laboratory Technicians and Data Entry staff are responsible for sample storage, data entry, and inventory verification, while Senior Technicians perform verification in the informatics system.

ABBREVIATIONS

Ca	Cancer
OT	Operation Theatre
CT	Chemotherapy
HT	Hormone Therapy
Post NACT	Post-Neoadjuvanvt Chemotherapy
POST RT	Post Radiotherapy
BC	Buffy Coat
PP	Plasma
NT	Normal Tissue
TT	Tumour Tissue
UIN	Unique Identification Number
Rpm	Revolution Per Minute
DNA	Deoxyribo Nucleic Acid
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MRD	Medical Record Department
EDTA	Ethylenediaminetetra acetic Acid
BRC	Bio samples Release Committee
PAC	Pre Anesthetic Check up
QC	Quality Control
bp	base pairs
DMSO	Dimethyl Sulfoxide
FCS	Fetal Calf Serum
PBMC	Peripheral Blood Mononuclear Cells
RT	Room Temperature (18°-25°C)
EDTA	Ethylene Diamine tetra acetic acid
ACD	Acid citrate dextrose

1.0 PURPOSE

In operating a Biobank there is a responsibility to maintain and operate the biobank to safeguard the collection. The use of an informatics system for documenting and tracking the collection is crucial. A database developed specifically for documenting and storing sample information will be part of the informatics system. As part of the Quality Assurance system, inventory verification should be conducted to confirm that the appropriate specimens are in the correct freezer locations.

2.0 SCOPE

This SOP covers the procedures for inventory verification. It outlines process validation steps to be followed to check that the correct storage locations have been entered in the computerized inventory system.

3.0 ROLES AND RESPONSIBILITIES

This SOP applies to all personnel of NLDB that are responsible for obtaining, processing storing and tracking human biological samples in the bank.

Laboratory Technician/Technologist, Data Entry - Responsible for storing samples, entering data in the informatics system and for conducting inventory verification

Senior Technician: Responsible for conducting verification on informatics system

4.0 MATERIALS, EQUIPMENT AND FORMS

- Inventory Database
- Safety equipment for handling stored samples such as face shield and thermal gloves for liquid nitrogen storage containers.

5.0 PROCEDURES

The primary purpose of the informatics system is to annotate and track inventory within a biobank or biobank network. This verification procedure is designed to confirm that appropriate biobank samples are in the correct location in the storage unit as indicated by the computerized inventory system (eg. cabinets, refrigerators, freezers, liquid nitrogen tanks). It validates that procedures are working to ensure sample traceability.

5.1 Verification Procedures – Personnel and Timing

- Assign biobank personnel qualified by training and education to conduct the Verification.
- Ensure that the assigned biobank personnel have authority access to the informatics system and storage facility

5.2 Verification of Inventory

- Conduct inventory audit on a periodic basis and sample selection for inventory verification on a random basis
- Ensure that a percentage of new samples collected are included since the last time inventory verification was performed.
- Remove sample from storage receptacle and verify that label matches the sample recorded in the database
- Minimize time that samples are handled or removed from required storage conditions
- Ensure that the temperature is controlled during inventory verification.
- Return sample to its designated storage spot and ensure that storage unit reaches optimally set temperatures.
- Document results of inventory verification.
- If sample is missing or incorrect, change inventory system to reflect the actual situation.

6.0 APPLICABLE REFERENCES, REGULATIONS AND GUIDELINES

- 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
- US National Biospecimen Network Blueprint. http://biospecimens.cancer.gov/resources/publications/reports/nbn.asp
- Human Tissue and Biological Samples for use in Research. Operational and Ethical Guidelines. Medical Research Council Ethics. http://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC002420

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Standard Operating Procedure

DOCUMENT NAME: MATERIALS HANDLING AND DOCUMENTATION

DOCUMENT NO. :ILBS#NLDB:H

Document Name : Blood collection
Document No. : ILBS#NLDB:H.4
Version No. : 1.0
Effective Date : 01/01/2025

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MATERIALS HANDLING AND DOCUMENTATION

Blood collection

Number	Effective date	Pages	Author	Authorized by
ILBS#NLDB:H.4	01/01/2025	9	Mr. Satish Kumar	Dr.Chhagan Bihari
Version	**Review period**	**No. of copies**	**Approved by**	**Date**
1.0	2yrs	3	Dr.Chhagan Bihari	30/12/2024

Location	Subject
Biobank reception area Almira	Blood collection
Function	**Distribution**
This SOP gives information about the proper collection, labelling, handling, and transportation of whole blood samples for biobank processing and storage.	➢ HOD ➢ Biobank Reception area ➢ Master files

SCOPE AND APPLICATION:

This SOP describes the procedure for collection of whole blood samples for processing and storage in the biobank in accordance with established biosafety and health and safety requirements.

RESPONSIBILITY:

NLDB technicians and its collaborative centres are responsible for performing venipuncture to obtain blood from the consented participant.

ABBREVIATIONS

Ca	Cancer
OT	Operation Theatre
CT	Chemotherapy
HT	Hormone Therapy
Post NACT	Post-Neoadjuvanvt Chemotherapy
POST RT	Post Radiotherapy
BC	Buffy Coat
PP	Plasma
NT	Normal Tissue
TT	Tumour Tissue
UIN	Unique Identification Number
Rpm	Revolution Per Minute
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FCS	Fetal Calf Serum
PBMC	Peripheral Blood Mononuclear Cells
RT	Room Temperature (18°-25°C)
EDTA	Ethylene Diamine tetra acetic acid
ACD	Acid citrate dextrose

1.0 PURPOSE

Blood samples are drawn from patients that have been through the informed consent process and agreed to participate in the NLDB biobank program. The objective of this procedure is to define the course of action and to establish the basic quality guidelines with respect to collecting and handling and to the processing of whole blood samples that will be deposited in NLDB or any center or hospital affiliated to the biobank.

2.0 SCOPE

This procedure applies to all whole blood samples that are extracted in order to be processed and stored in a biobank. This protocol does not detail the occupational health and safety processes regarding biohazardous materials and/or chemical products, and it is recommended that the personnel must follow the Health and Safety rules established in each center.

3.0 ROLES AND RESPONSIBILITIES

The SOP applies to all personnel from NLDB and its collaborative centres that are responsible for performing venipuncture to obtain blood from the consented participant.

4.0 ABBREVIATIONS

- **EDTA:** Ethylene diaminetetra acetic acid. EDTA attracts calcium ions, thus blocking the coagulation cascade (di-potassium salt K2, or K3).
- **ACD:** Citric acid, citrate and dextrose in amounts of 0.9, 2, and 2 g, respectively, in 120 ml of distilled water. It is used to obtain plasma for coagulation and platelet function assays. It is used in collection and storage for transfusion since it preserves the blood longer, particularly the survival of erythrocytes: 21-32 days-70% survival. It changes the calcium concentration.
- **CPD:** Citrate - Phosphate -Dextrose.
- **CPD-A:** Citrate - Phosphate –Dextrose-Adenine

5.0 DEFINITIONS

- Anticoagulant: a substance that prevents blood clotting.
- Lipemia: presence of lipids (cholesterol, triglycerides and phospholipids) in the blood, which in normal conditions usually ranges from 400 to 700 mg per 100 ml of blood.
- Jaundice: too much bilirubin in the blood to be removed.

6.0 MATERIALS & SUPPLIES

- Syringes and/or material required for collecting blood.
- Blood collection tube for haemogram and/or haematology, with:

- EDTA as anticoagulant (purple stopper). (In case determination of metals like lead, cadmium, zinc etc. is required, the tube with anticoagulant should be free of these metals, as well as all material that comes into contact with the blood).
- Gloves to protect staff who are handling blood and/or biohazardous materials.
- Lab coat to protect against spills and spatters.
- Sufficient and appropriate labels for collection tubes.
- Citrate as anticoagulant (yellow stopper)
- Heparin as an anticoagulant (green stopper)
- Sterile Pasteur pipettes
- Blood collection tube racks
- Cryo-tubes racks
- 1.5 - 2ml sterile cryo-tubes
- Markers and ballpoints
- Filter paper
- Label printer
- Cryo storage boxes
- Ultra-freezer -80°C

7.0 PROCEDURES

7.1 Coordination for the Collection and Processing of Blood Samples

7.1.1 Blood must be collected after the patient has signed the informed consent for donating samples to the biobank. It is recommended that the time between blood collection and freezing at -80ºC be defined based on the type of studies for which the sample is intended.

a) Optimum time for cell studies: maximum 1.5 hours after extraction; and
b) Optimum time for virological studies: maximum 24 hours after extraction.

7.1.2 Blood is collected via peripheral venipuncture. The people in charge of carrying out this procedure and of programming extractions must to ensure that the blood collection tubes with anticoagulants are properly identified and that a proper collection and reception of the sample is guaranteed.

7.1.3 It is advised to take the maximum possible information concerning the sample at the time of extraction:

- Date and time of withdrawal.
- Type of anticoagulant.
- Incidents not related to the protocol.

7.2 Preparation:Verification and Identification of the Tubes

7.2.1 Personnel qualified by training to draw blood must perform blood collection.

7.2.2 Start filling out the blood collection worksheet. Identify the patient, verify identification, andcheck that informed consent has been obtained.

7.2.3 Assess patient's physical and mental disposition and determine if this is the appropriate timeto draw blood.

7.2.4 Be courteous, professional, and sensitive to the patient's needs. Ensure that allCommunications are discreet and respectful of patient confidentiality.

7.2.5 Assemble proper equipment to draw blood.

7.2.6 Check patient information, while always maintaining privacy and ethics as on the Protection of Personal Data, and other relevant national laws applicable to this process, and ensure the correct relationship between the properly labelled blood collection tubes and patient information.

7.2.7 Label a blood collection tube containing anticoagulant to obtain whole blood aliquots. The type of anticoagulant used must be the most appropriate one for studies for which the sample is intended. Based on previous experience, the following is advised:

- Cell proliferation studies - leukocytes: Heparin.
- DNA extraction: EDTA.
- Cryopreservation of red blood cells: ACD, CPD-A or CPD

7.3 Drawing & Collection of Blood

7.3.1 Provide for the patient's comfort as much as possible, and gain the patient's cooperation.Position the patient. The patient should sit in a chair, lie down or sit up in bed. Hyperextendthe patient's arm.

7.3.2 Apply tourniquet to expose veins. Do not place too tightly. If superficial veins are not easilyapparent, force blood into the vein by massaging the arm from wrist to elbow, tap the sitewith index and second finger, apply a warm, damp cloth to the site or lower extremity to allowveins to fill.

7.3.3 Select appropriate site for venipuncture. Avoid areas with excessive scars or hematomas.While hand and wrist veins are acceptable it is optimal to select an antecubital vein.

7.3.4 Prepare the patient's arm using alcohol swab. Cleanse in a circular fashion, beginning at thesite and working outward. Allow to air dry.

7.3.5 Anchor the vein and swiftly insert the needle (at a 15-30 degree angle with the surface of thearm) into the lumen of the vein. Avoid excessive probing and trauma to the site.

7.3.6 Draw blood (as recommended minimum volume) into an evacuated blood collectiontube. Fill in additional appropriate tubes for DNA and RNA extraction if relevant.

7.3.7 When the last tube to be drawn is filling, remove the tourniquet.

7.3.8 Remove the needle from the patient and apply a gauze and adequate pressure to the site ofvenipuncture to avoid hematoma formation.

7.3.9 Dispose of needles and supplies in a safe manner.

7.3.10 Immediately after extraction, gently invert the tube 6-8 times to mix the blood well with the corresponding anticoagulant.

7.3.11 Transport the blood to the laboratory for processing in a time not exceeding the maximum time after extraction of the sample defined for each study, while following the safety guidelines for the transport of biological material established. The blood must be transferred to the biobank at room temperature, never cold.

7.3.12 In the biobank properly labelled and identified micro tubes for storage of whole blood must be prepared. Depending on the age, incidents or the physical condition of the patient, the volume of blood that is received may vary even though withdrawals should approximate the following reference values to allow its processing and use:

Table 1: Relationship age – extraction volume

AGE OF PATIENT	AVERAGE BLOOD VOLUME
0-2 YEARS	0.5 – 2 mL
3-5 YEARS	2.5 – 5 mL
6-11 YEARS	5-10 mL
> 12 YEARS	10-15 mL
> 18 YEARS	<25 mL

7.3.13 Transfer at least 0.5ml of whole blood to appropriately labelled and identified cryotube(s).

7.3.14 Place the micro-tubes in cryo storage boxes and store them in -80ºC freezer.

7.3.15 Record the location of the stored sample in the biobank software.

7.4 Maintaining Traceability and Data Associated to the Sample

It is advice to gather the maximum amount of information possible concerning the sample, both at the time of reception and after processing and storage, and depending on the studies for which they will be used, for example:

- Date and time of receipt and/or processing
- Degree of hemolysis
- Volume of blood received
- Degree of Lipemia
- Degree of jaundice
- Degree of coagulation
- Incidents during processing

8.0 APPLICABLE REFERENCES, REGULATIONS AND GUIDELINES

- Declaration of Helsinki. http://www.wma.net/en/30publications/10policies/b3/index.html
- Tri-Council Policy Statement 2; Ethical Conduct for Research Involving Humans; Medical ResearchCouncil of Canada; Natural Sciences and Engineering Council of Canada; Social Sciences andHumanities Research Council of Canada, December 2010. http://www.pre.ethics.gc.ca/eng/policy-politique/initiatives/tcps2-eptc2/Default/
- Human Tissue and Biological Samples for use in Research. Operational and Ethical Guidelines.Medical Research Council Ethics http://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC002420
- Best Practices for Repositories I. Collection, Storage and Retrieval of Human Biological Materials forResearch. International Society for Biological and Environmental Repositories (ISBER). http://www.isber.org/Search/search.asp?zoom_query=best+practices+for+repositories
- US National Biospecimen Network Blueprint http://biospecimens.cancer.gov/resources/publications/reports/nbn.asp
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- http://www.ctrnet.ca/resources/operating-procedures
- Whole Blood Collection: https://brd.nci.nih.gov/brd/sop/download-pdf/1066

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Standard Operating Procedure

DOCUMENT NAME: MATERIALS HANDLING AND DOCUMENTATION

DOCUMENT NO. :ILBS#NLDB:H

MATERIALS HANDLING AND DOCUMENTATION

Blood Processing and Storage

Document Name : Blood Processing and Storage
Document No. : ILBS#NLDB:H.5
Version No. : 1.0
Effective Date : 01/01/2025

Address

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MATERIALS HANDLING AND DOCUMENTATION

Blood Processing and Storage

Number	Effective date	Pages	Author	Authorized by
ILBS#NLDB:H.5	01/01/2025	8	Mr. Satish Kumar	Dr.Chhagan Bihari
Version	**Review period**	**No. of copies**	**Approved by**	**Date**
1.0	2yrs	3	Dr. Chhagan Bihari	30/12/2024

Location	Subject
Biobank Reception area Almira	Blood Processing and Storage
Function	**Distribution**
To give information about blood processing and storage.	➢ HOD ➢ Biobank Reception area ➢ Master files

SCOPE AND APPLICATION:

This SOP describes the procedure for processing, labelling, storage, blood samples and blood-derived components to ensure sample integrity, traceability, and biosafety.

RESPONSIBILITY:

NLDB technicians are responsible for proper handling, processing, transport, labelling, and storage of blood samples as per established procedures.

ABBREVIATIONS

Ca	Cancer
OT	Operation Theatre
CT	Chemotherapy
HT	Hormone Therapy
Post NACT	Post-Neoadjuvanvt Chemotherapy
POST RT	Post Radiotherapy
BC	Buffy Coat
PP	Plasma
NT	Normal Tissue
TT	Tumour Tissue
UIN	Unique Identification Number
Rpm	Revolution Per Minute
DNA	Deoxyribo Nucleic Acid
RNA	Ribo Nucleic Acid
MRD	Medical Record Department
EDTA	Ethylenediaminetetra acetic Acid
BRC	Bio samples Release Committee
PAC	Pre Anesthetic Check up
QC	Quality Control
bp	base pairs
DMSO	Dimethyl Sulfoxide
FCS	Fetal Calf Serum
PBMC	Peripheral Blood Mononuclear Cells
RT	Room Temperature (18°-25°C)
EDTA	Ethylene Diamine tetra acetic acid
ACD	Acid citrate dextrose

1.0 PURPOSE

Blood samples are drawn from patients/donors that have been through the informed consent process and agreed to participate in the NLDB program. Blood samples are obtained by personnel qualified to draw blood from patients/donors in the hospitals or other collection centres. Processed blood products are an important biobank resource.

2.0 SCOPE

This SOP describes how blood should be processed, accessioned and stored.

3.0 ROLES AND RESPONSIBILITIES

The SOP applies to all laboratory personnel from NLDB biobank that are responsible for the processing of blood to obtain blood products for storage in the NLDB biobank. It also applies to personnel responsible for collection of the blood from the consented participant/donor.
Technician: Transport, process and store blood and blood products

4.0 MATERIALS, EQUIPMENT AND FORMS

- Evacuated blood collection tubes for plasma (e.g. Lavender top tube with EDTA)
- Evacuated blood collection tubes (e.g. tube for separating serum)
- Tube for extraction of nucleic acids from blood (e.g. Paxgene tube)
- Filter product for easy long term DNA storage (e.g. Whatman FTA Elute)
- Blood Collection/Processing Worksheets
- Aerosol-resistant pipette tips
- Cryo-tubes
- Transfer pipettes
- Storage boxes
- Centrifuge
- Pipettors
- Gloves
- Racks
- Labels

5.0 PROCEDURES

This procedure is intended to ensure that blood samples obtained from consented participants/donors are processed in a safe and efficient manner while eliminating the risks of contamination and loss.

5.1 Timing for Blood Collection and Processing

5.1.1 Preferably, blood collection should be done pre-operation and as close as possible to the time when the tissue is donated to the biobank or at an alternative time, if appropriate for the research study.

5.1.2 The time requirement for sample processing depends on the intended use and therefore, time to processing should be recorded.

5.2 Verification of Identification Information on Tubes

As applicable, verify the accuracy of patient information (in keeping with privacy and ethical policies) and ensure that it corresponds with the information on labels on blood collection tubes.

5.3 Accessioning of Samples

Accession of plasma, serum and buffy coat samples into biobank inventory database as per procedure for the site-specific inventory system and affix appropriate labels on the vials.

5.4 Separation of Plasma from the Cellular Fraction

The whole blood can be processed directly for DNA or RNA, or processed as described below to obtain a buffy coat fraction and plasma for cryopreservation.

5.4.1 In the area designated by the biobank for processing blood, fractionate the whole blood (blood collected in tubes containing an anticoagulant such as ethylene-diamineteraacetic acid-EDTA or Heparin) by centrifuging at 1500-2000 x g for 15 minutes at room temperature.

This will separate the blood into three visible layers (see Figure 1):

a. The upper layer is generally clear and pale yellow in colour.

b. The second layer is a narrow greyish white interface band representing the “buffy coat” or leukocyte fraction.

c. The third or bottom layer is dark red and consists of the erythrocytes or red blood cells.

5.4.2 Using an appropriate disposable transfer pipette, aspirate off the plasma layer down to approximately 1 mm from the buffy coat layer. Take care not to disturb the leukocyte or buffy coat layer.

5.4.3 Expel all plasma from the pipette into a plasma collection tube.

5.4.4 Aliquot recovered plasma and place into labelled cryovials.

5.4.5 Place the cryovials in appropriate storage units. For long-term storage, -80° C or colder is recommended.

5.4.6 Record position and location of the tubes.

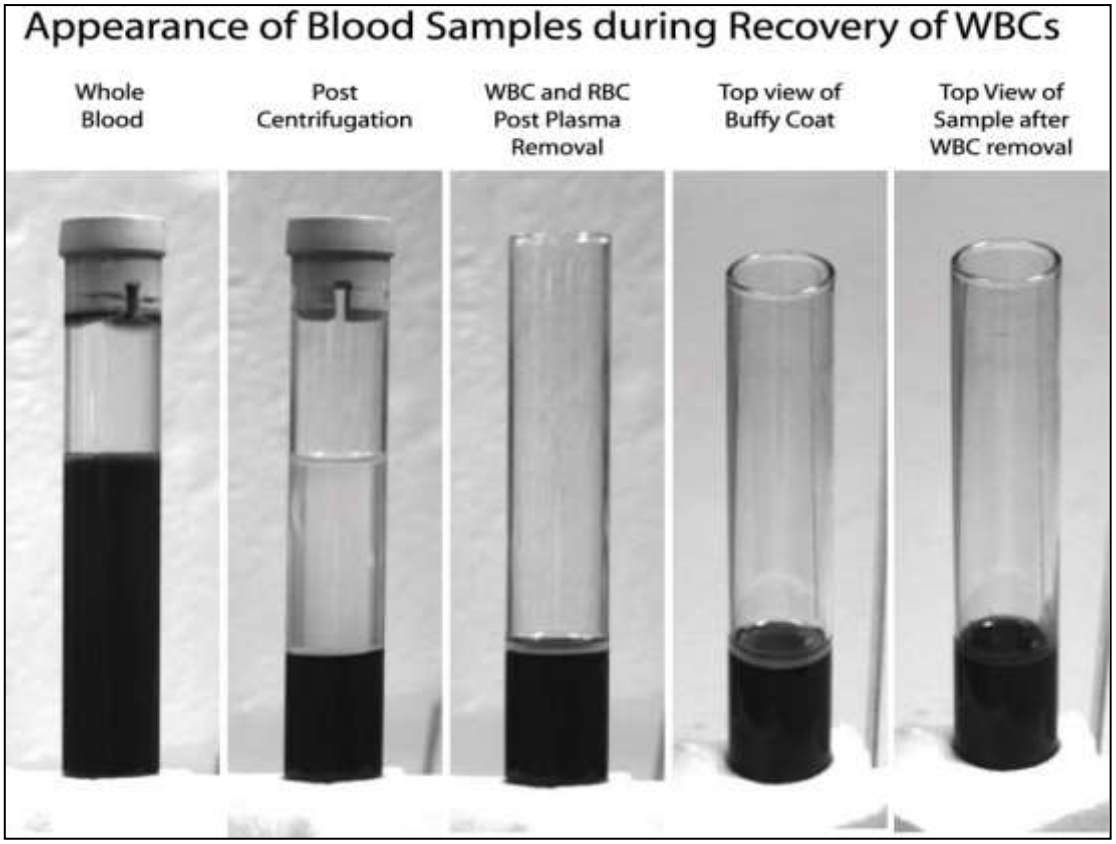

Figure 2: Blood Samples during WBC Recovery

5.5 Recovery of White Blood Cells

5.5.1 After removing the plasma layer, use a transfer pipette to aspirate the entire buffy coat layer (usually a volume of 0.5 mL or less).

5.5.2 Expel the buffy coat into a single cryovial. Triturate the sample, and then take half the sample and store in a separate cryovial.

5.5.3 Place the cryovials in appropriate storage units. For long-term storage, -80° C or colder is recommended.

5.5.4 Record position and location of the tubes.

5.6 Separation of Serum from Blood Samples

5.6.1 If serum is to be obtained from the blood samples, collect the blood in appropriate tubes.

5.6.2 For non-gel serum tubes, invert the tubes 8 times immediately following collection to ensure proper coagulation.

5.6.3 Incubate to ensure complete coagulation. It is recommended to process within 1 hour.

5.6.4 Prepare the required amount of 2 ml cryovials to be used for storage of the serum with the relevant labels on each tube.

5.6.5 Following incubation, centrifuge the serum tubes at 1500 g for 15 minutes.

5.6.6 Aspirate the supernatant and transfer directly to the labelled cryovials.

5.6.7 Place the cryovials in appropriate storage units. For long-term storage, -80° C or colder is recommended.

5.6.8 Record the position and location of the vials in the storage container.

5.7 Alternate Processing of Blood Samples

If blood has been collected in specific collection tubes for extraction of DNA or RNA then proceed with processing of these collection tubes as per established procedures for DNA and RNA processing and extraction.

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Standard Operating Procedure

DOCUMENT NAME: MATERIALS HANDLING AND DOCUMENTATION

DOCUMENT NO. :ILBS#NLDB:H

Document Name : Blood Derivatives: Extraction of RNA
Document No. : ILBS#NLDB:H.6
Version No. : 1.0
Effective Date : 01/01/2025

Address
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MATERIALS HANDLING AND DOCUMENTATION

Blood Derivatives: Extraction of RNA

Number	Effective date	Pages	Author	Authorized by
ILBS#NLDB:H.6	01/01/2025	7	Mr. Satish Kumar	Dr.Chhagan Bihari
Version	**Review period**	**No. of copies**	**Approved by**	**Date**
1.0	2yrs	3	Dr. Chhagan Bihari	30/12/2024

Location	Subject
Biobank Reception area almira	Blood Derivatives: Extraction of RNA
Function	**Distribution**
This SOP gives information about the extraction of high-quality RNA from blood and tissue samples for downstream biobank applications.	➢ HOD ➢ Biobank Reception area ➢ Master files

SCOPE AND APPLICATION:

This SOP describes RNA extraction from snap-frozen and OCT-embedded tissue samples for biobank use. Personnel must follow institutional biosafety guidelines.

RESPONSIBILITY:

NLDB Laboratory technicians/technologists are responsible for sample labelling and RNA extraction procedures

ABBREVIATIONS

Ca	Cancer
OT	Operation Theatre
CT	Chemotherapy
HT	Hormone Therapy
Post NACT	Post-Neoadjuvanvt Chemotherapy
POST RT	Post Radiotherapy
BC	Buffy Coat
PP	Plasma
NT	Normal Tissue
TT	Tumour Tissue
UIN	Unique Identification Number
Rpm	Revolution Per Minute
DNA	Deoxyribo Nucleic Acid
RNA	Ribo Nucleic Acid
MRD	Medical Record Department
EDTA	Ethylenediaminetetra acetic Acid
BRC	Bio samples Release Committee
PAC	Pre Anesthetic Check up
QC	Quality Control
bp	base pairs
DMSO	Dimethyl Sulfoxide
FCS	Fetal Calf Serum
PBMC	Peripheral Blood Mononuclear Cells
RT	Room Temperature (18°-25°C)
EDTA	Ethylene Diamine tetra acetic acid
ACD	Acid citrate dextrose

1.0 PURPOSE

Genomic studies often utilize nucleic acids (DNA and RNA) derived from patient samples. When extracting and storing ribonucleic acid **(**RNA) from blood samples all efforts should be made to avoid contamination, prevent degradation and preserve molecular integrity. RNA degradation is a major problem during the collection, processing, and storage of clinical samples. The purpose of this document is to outline standardized procedures for NLDB biobanks to follow when extracting RNA from blood samples.

2.0 SCOPE

This standard operating procedure (SOP) describes how RNA should be extracted from blood samples. The SOP does not cover detailed safety procedures for handling Human Biological Materials (HBMs) or hazardous chemicals and it is recommended that personnel follow institutional safety guidelines.

3.0 ROLES AND RESPONSIBILITIES

The SOP applies to all personnel from NLDB biobank that are responsible for extracting RNA from blood.

Bio Bank Personnel	**Responsibility / Role**
Laboratory Technician/Technologist	Responsible for labeling tubes and extracting RNA from blood samples.

4.0 MATERIALS, EQUIPMENT AND FORMS

The materials, equipment and forms listed in the following list are recommendations only and may be substituted by alternative/equivalent products more suitable for the site-specific task or procedure.

Materials and Equipment	
Markers, ink and pens	
Appropriate labels for tubes and vials	
RNA extraction Kit and instructions	
Biohazardous waste container and autoclave bags	
Storage Boxes	
RNase inhibitor to clean surfaces	

5.0 PROCEDURES

This procedure is intended to ensure that RNA is extracted from blood samples in a safe and consistent manner while eliminating the risks of contamination and loss of

molecular and structural integrity. Consistency in procedure is important for obtaining comparable and reliable test results.

5.1 Extraction of RNA from Blood Samples – General Extraction Considerations

5.1.1 Avoiding Cross Contamination

a. Due to the sensitivity of nucleic acid amplification technologies, precautions should be taken to avoid cross contamination of samples.

b. Many RNA extraction protocols use spin columns. Avoid moistening the rim of the spin columns with pipette tips and avoid touching the column with the pipette tip.

c. Always use aerosol-barrier tips.

d. Avoid cross-contamination after each vortexing step. Briefly centrifuge the tubes to remove droplets that may be on the lids of the tubes.

e. Close the lids of the spin columns before placing in the microcentifuge.

f. Flow-through generated after each centrifugation step may contain hazardous materials and should be disposed of appropriately.

g. Only open one spin column at a time and avoid creating aerosols.

h. Discard used processing tubes containing flow-through into appropriate biohazardous waste containers

5.1.2 Avoiding Degradation of RNA

a. Work on clean surfaces and when appropriate wipe down with an RNase inhibitor.

b. Do not use any plastic-ware or glassware without first eliminating RNase contamination.

c. Take care not to introduce RNase into the sample during or after the purification procedure.

d. It is optimal to use sterile RNase free disposable vessels and solutions while working with RNA. Microbiological aseptic technique is always optimal to use when working with RNA.

e. Wear latex or vinyl gloves while handling reagents, tubes, and samples to prevent RNase contamination from the skin or surface of the laboratory.

f. Change gloves frequently.

g. Keeps tubes closed whenever possible.

h. Keep purified RNA on wet ice for processing and for aliquoting.

i. Keep samples frozen below -80º C or lower for long-term storage.

5.2 Extraction of RNA from Blood Samples

5.2.1 Treat all blood as potentially infectious.

5.2.2 RNA extraction is performed by the laboratory technician/technologist or trained personnel designated by the NLDB.

5.2.3 Have materials and equipment ready. Have as many tubes and cryovials as needed labelled and ready.

5.2.4 Document the method of RNA extraction. There are several commercially available RNA extraction kits available, follow the detailed procedure outlined in the appropriate commercial kit handbook.

5.2.5 Immediately after the procedure, place extracted and re-suspended RNA on ice.

5.2.6 It is preferable to aliquot RNA into several smaller aliquots to limit freeze thaw cycles.

5.2.7 A small aliquot should be removed prior to long-term storage for quantitation and/or quality control.

5.2.8 Place extracted RNA samples in storage boxes and record location

5.2.9 Place samples at -80º C or lower.

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Standard Operating Procedure

DOCUMENT NAME: MATERIALS HANDLING AND DOCUMENTATION

DOCUMENT NO. :ILBS#NLDB:H

Document Name	**:**	**Blood Derivatives: Extraction of DNA**
Document No.	**:**	**ILBS#NLDB:H.7**
Version No.	**:**	**1.0**
Effective Date	**:**	**01/01/2025**

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MATERIALS HANDLING AND DOCUMENTATION

Blood Derivatives: Extraction of DNA

Number	Effective date	Pages	Author	Authorized by
ILBS#NLDB:H.7	01/01/2025	7	Mr. Satish Kumar	Dr.Chhagan Bihari
Version	**Review period**	**No. of copies**	**Approved by**	**Date**
1.0	2yrs	3	Dr. Chhagan Bihari	30/12/2024

Location	Subject
Biobank Reception area Almira	Blood Derivatives: Extraction of DNA
Function	**Distribution**
This SOP gives information about the extraction of high-quality DNA from blood samples for biobank processing and downstream applications.	➢ HOD ➢ Biobank Reception area ➢ Master files

SCOPE AND APPLICATION:

This SOP describes the procedure for extraction of DNA from blood samples for biobank processing and storage while following institutional biosafety guidelines.

RESPONSIBILITY:

NLDB Laboratory technicians are responsible for sample labelling and DNA extraction procedures.

ABBREVIATIONS

Ca	Cancer
OT	Operation Theatre
CT	Chemotherapy
HT	Hormone Therapy
Post NACT	Post-Neoadjuvanvt Chemotherapy
POST RT	Post Radiotherapy
BC	Buffy Coat
PP	Plasma
NT	Normal Tissue
TT	Tumour Tissue
UIN	Unique Identification Number
Rpm	Revolution Per Minute
DNA	Deoxyribo Nucleic Acid
RNA	Ribo Nucleic Acid
MRD	Medical Record Department
EDTA	Ethylenediaminetetra acetic Acid
BRC	Bio samples Release Committee
PAC	Pre Anesthetic Check up
QC	Quality Control
bp	base pairs
DMSO	Dimethyl Sulfoxide
FCS	Fetal Calf Serum
PBMC	Peripheral Blood Mononuclear Cells
RT	Room Temperature (18°-25°C)
EDTA	Ethylene Diamine tetra acetic acid
ACD	Acid citrate dextrose

1.0 PURPOSE

Tissue samples are collected from patients that have been through the informed consent process and agreed to participate in the NLDB biobank program. Genomic studies often utilize nucleic acids (DNA and RNA) derived from these samples. When extracting and storing deoxyribonucleic acid (DNA) from blood samples all efforts should be made to avoid contamination, prevent degradation and preserve molecular integrity. The purpose of this document is to outline standardized procedures for NLDB biobank to follow when extracting DNA from blood samples.

2.0 SCOPE

This standard operating procedure (SOP) describes how DNA should be extracted from blood samples. The SOP does not cover detailed safety procedures for handling Human Biological Materials (HBMs) or hazardous chemicals and it is recommended that personnel follow institutional safety guidelines.

3.0 ROLES AND RESPONSIBILITIES

The SOP applies to all personnel from NLDB biobank that are responsible for extracting RNA from blood.

Bio Bank Personnel	**Responsibility / Role**
Laboratory Technician/Technologist	Responsible for labeling tubes and extracting DNA from blood samples.

4.0 MATERIALS, EQUIPMENT AND FORMS

The materials, equipment and forms listed in the following list are recommendations only and may be substituted by alternative/equivalent products more suitable for the site-specific task or procedure.

Materials and Equipment	
Markers, ink and pens	
Appropriate labels for tubes and vials	
Tube of previously isolated Buffy Coat from blood sample	
Biohazardous waste container and autoclave bags	
2 ml Microfuge tubes	
1.5 ml centrifuge tubes	
2 ml cryotubes	
Racks for microfuge tubes	

Tube racks for water bath	
Vortex mixer	
Microcentrifuge	
Pipettes	
Sterile pipette tips with aerosol barrier	
Transfer pipettes	
Micropipettors	
Isopropanol	
DNA Extraction Kit	
A shaking Heat Block like the EppendorfThermomixer or normal heating block or water bath.	
-80º C and -20º freezer	
Storage boxes	
Disposable gloves	
Hot water bath (set at 55° C)	
Tube racks for water bath	
Rolling rack (rotator mixer)	
95% ethanol	
70% ethanol	
Refrigerator at 4° C	

5.0 PROCEDURES

This procedure is intended to ensure that DNA is extracted from blood samples in a safe and consistent manner while eliminating the risks of contamination and loss of molecular and structural integrity. Consistency in procedure is important for obtaining comparable and reliable test results. Work with tubes in the same order for the duration of the procedure to minimize the potential for sample mix up.

5.1 Extraction of DNA from Blood Samples Using a Column Based Kit

5.1.1 Document the method of DNA extraction. There are several commercially available DNA extraction kits available, follow the detailed procedure outlined in the appropriate commercial kit handbook.

5.1.2 Treat all blood as potentially infectious.

5.1.3 DNA extraction is performed by the laboratory technician/technologist or trained personnel designated by the NLDB biobank.

5.1.4 Have materials and equipment ready. Have as many tubes and cryovials as needed labelled and ready.

5.1.5 Thaw the previously frozen Buffy Coat by gentle agitation in a 37º C water bath.

5.1.6 Keep the thawed tube on ice until starting the extraction procedure.

5.1.7 Use a DNA extraction kit, follow protocols, and document

5.1.8 Genomic DNA can be stored at 4ºC.

5.1.9 Quantitate DNA by spectophotometry and/or fluorometry. Record concentration on stock tube.

5.1.10 DNA is a weak acid and at 4ºC may be subject to acid hydrolysis. For long-term storage keeps DNA at -80º C. Avoid subjecting the DNA to freeze/thaw cycles to prevent fragmentation of the genomic DNA.

5.1.11 Place DNA in storage boxes and record storage location.

5.2 Data Elements to Track

5.2.1 Source sample ID and type (whole blood, buffy coat, etc.) and volume

5.2.2 DNA sample Id.

5.2.3 Date extracted

5.2.4 DNA extraction method

5.2.5 DNA volume

5.2.6 DNA concentration and quantitation method

5.2.7 Ratio of A260nm/A280nm

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Standard Operating Procedure

DOCUMENT NAME: MATERIALS HANDLING AND DOCUMENTATION

DOCUMENT NO. :ILBS#NLDB:H

Document Name : DNA Extraction from Blood
Document No. : ILBS#NLDB:H.8
Version No. : 1.0
Effective Date : 01/01/2025

Address
National Liver Disease Biobank,
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MATERIALS HANDLING AND DOCUMENTATION

DNA Extraction from Blood

Number	Effective date	Pages	Author	Authorized by
ILBS#NLDB:H.8	01/01/2025	9	Mr. Satish Kumar	Dr.Chhagan Bihari
Version	**Review period**	**No. of copies**	**Approved by**	**Date**
1.0	2yrs	3	Dr. Chhagan Bihari	30/12/2024

Location	Subject
Biobank Reception area Almira	DNA Extraction from Blood
Function	**Distribution**
To give information about DNA Extraction from Blood.	➢ HOD ➢ Biobank Reception area ➢ Master files

SCOPE AND APPLICATION:

This SOP describes the procedure for extraction of DNA from blood samples. Personnel handling human biological materials and hazardous chemicals shall follow institutional safety guidelines.

RESPONSIBILITY:

NLDB technicians are responsible for extracting DNA from blood.

ABBREVIATIONS

Ca	Cancer
OT	Operation Theatre
CT	Chemotherapy
HT	Hormone Therapy
Post NACT	Post-Neoadjuvanvt Chemotherapy
POST RT	Post Radiotherapy
BC	Buffy Coat
PP	Plasma
NT	Normal Tissue
TT	Tumour Tissue
UIN	Unique Identification Number
Rpm	Revolution Per Minute
DNA	Deoxyribo Nucleic Acid
RNA	Ribo Nucleic Acid
MRD	Medical Record Department
EDTA	Ethylenediaminetetra acetic Acid
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FCS	Fetal Calf Serum
PBMC	Peripheral Blood Mononuclear Cells
RT	Room Temperature (18°-25°C)
EDTA	Ethylene Diamine tetra acetic acid
ACD	Acid citrate dextrose

1.0 PURPOSE

Tissue samples are collected from patients that have been through the informed consent process and agreed to participate in the NLDB biobank program. Genomic studies often utilize nucleic acids (DNA and RNA) derived from these samples. When extracting and storing DNA from blood samples all efforts should be made to avoid contamination, prevent degradation and preserve molecular integrity. The purpose of this document is to outline standardized procedures for NLDB biobank to follow when extracting DNA from white blood cells obtained from 5 to 10 mls of whole blood samples (EDTA or ACD) using the phenol/chloroform (organic solvent) method.

2.0 SCOPE

This standard operating procedure (SOP) describes how DNA should be extracted from blood samples. The SOP does not cover detailed safety procedures for handling Human Biological Materials (HBMs) or hazardous chemicals and it is recommended that personnel follow institutional safety guidelines.

3.0 ROLES AND RESPONSIBILITIES

The SOP applies to all personnel from NLDB biobank that are responsible for extracting DNA from blood.

Bio Bank Personnel	**Responsibility / Role**
Laboratory Technician/Technologist	Responsible for labeling tubes and extracting DNA from blood samples.

4.0 MATERIALS, EQUIPMENT AND FORMS

The materials, equipment and forms listed in the following list are recommendations only and may be substituted by alternative/equivalent products more suitable for the site-specific task or procedure.

Materials and Equipment	
Markers, ink and pens	
Appropriate labels for tubes and vials	
Tube of previously isolated white blood cells from 5-10 mls of whole blood sample	
14 ml polypropylene tube	
1.5 ml centrifuge tubes	
2 ml cryotubes	
Transfer pipette (3 ml)	
Racks for 1.5 ml centrifuge tubes	

Centrifuge for 14 ml tubes	
Racks for 14 ml tubes	
Microcentrifuge	
Sterile pipette tips with aerosol barrier	
Micropipettors	
Rolling rack to mix purified DNA	
10 ml glass pipettes for transferring phenol and chloroform (do not use polystyrene)	
Pipette gun for 10 ml glass pipettes	
-80º C and -20º freezer	
Storage boxes	
Disposable gloves	
Water bath (set at 60° C)	
Tube racks for water bath for 14 and 2 ml tubes	
Rolling rack (rotator mixer)	
95% ethanol - ice cold	
70% ethanol - ice cold	
Refrigerator at 4° C	
Lysis solution*	
Proteinase K 20 mg/ml	
TRIS Buffer saturated Phenol	
Chloroform /Isoamyl alcohol*	
5 M NaCl	
TRIS EDTA (TE) Buffer pH 8.0	

5.0 PROCEDURES

This procedure is intended to ensure that DNA is extracted from blood samples in a safe and consistent manner while eliminating the risks of contamination and loss of molecular and structural integrity. Consistency in procedure is important for obtaining comparable and reliable test results.

5.1 Extraction of DNA from Blood Samples using the Phenol/Chloroform Method

NOTE: Volumes indicated are recommendations only and should be scaled according to the size of the sample. It is possible to keep the supernatant at 4º C between each step.

5.1.1 Treat all blood as potentially infectious.

5.1.2 Phenol and chloroform are organic solvents and must be used in a fume hood. All phenol and chloroform waste must be discarded into the appropriate waste container for organic solvents.

5.1.3 DNA extraction is performed by the laboratory technician/technologist or trained personnel designated by the NLDB biobank.

5.1.4 Re-suspend white cell pellet in 2 ml of Lysis Buffer using a 3 ml transfer pipette.

5.1.5 Using the same transfer pipette, transfer cells and Lysis buffer to a 14 ml polypropylene screw cap tube. If pellet is smaller or larger than usual adjust volume of Lysis buffer accordingly.

5.1.6 Add 20 mg/ul Proteinase K to a final concentration of 200 ug/ml. Pipette up and down to mix using a 3 ml transfer pipette. To avoid shearing the genomic DNA do not vortex to mix.

5.1.7 Incubate 2-4 hours or overnight in a water bath at 60º C. Mix tube every 30 minutes when possible.

5.1.8 Add an equal volume of TRIS saturated Phenol.

5.1.9 Mix tube by rocking back and forth approximately 70 strokes per minute for at least 2 minutes.

5.1.10 Centrifuge at 900 g for 5 minutes at room temperature.

5.1.11 Label a new 14 ml tube.

5.1.12 Using a 1 ml pipettor, transfer upper phase (aqueous layer containing DNA) to the new 14 ml tube being careful not to remove the milky layer of the interphase.

5.1.13 Add an equal volume of 50:50 phenols: chloroform/Isoamyl alcohol.

5.1.14 Mix tube by rocking back and forth approximately 70 strokes per minute for at least 2 minutes.

5.1.15 Centrifuge at 900 g for 5 minutes at room temperature.

5.1.16 Label a new 14 ml tube.

5.1.17 Using a 1 ml pipettor, transfer upper phase to the new 14 ml tube being careful not to remove the milky layer of the interphase.

5.1.18 Repeat steps 13-17 for the second 50:50 phenol: chloroform/Isoamyl alcohol extraction.

5.1.19 Repeat steps 13-17 a third time if the interface is still very thick and milky.

5.1.20 Add an equal volume chloroform/Isoamyl alcohol.

5.1.21 Mix tube by rocking back and forth approximately 70 strokes per minute for at least 2 minutes.

5.1.22 Centrifuge at 900 g for 5 minutes at room temperature.

5.1.23 Label a new 14 ml tube.

5.1.24 Using a 1 ml pipettor, transfer upper phase to the new 14 ml tube. There should be little to no milky layer at the interphase.

5.1.25 Make the aqueous purified DNA 1M by using 5M NaCl. Mix gently.

5.1.26 Add 2.5 times the volume of ice cold 95% ethanol (stock kept at -20º C) and rock tube gently back and forth to precipitate out DNA.

5.1.27 Using a 1 ml pipette tip or 3 ml transfer pipette, transfer the DNA to a 1.5 ml microfuge tube.

5.1.28 Centrifuge at 180000 x g for 1 minute in a microfuge at room temperature.

5.1.29 Discard supernatant.

5.1.30 Add 500 ul of ice cold 70% ethanol and flick tube until DNA pellet dislodges from bottom of tube.

5.1.31 Centrifuge at 180000 x g for 1 min. in a microfuge at room temperature.

5.1.32 Discard supernatant.

5.1.33 Centrifuge at 180000 x g for 1 min. in a microfuge at room temperature. Discard supernatant.

5.1.34 Re-suspend DNA pellet in 500ul TE buffer. Adjust volume of TE buffer depending on the size of the DNA pellet.

5.1.35 Transfer DNA to a 2.0 ml cryovial for long term storage.

5.1.36 To ensure DNA is uniformly dissolved; Incubate at in 60 º C water bath and/or rotate overnight.

5.1.37 Store DNA at -80º C.

5.1.38 Discard all phenol and chloroform waste into the appropriate waste container for organic solvents.

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Standard Operating Procedure

DOCUMENT NAME: MATERIALS HANDLING AND DOCUMENTATION

DOCUMENT NO. :ILBS#NLDB:H

Document Name : Solid Tissue - Tissue Collection and Transportation
Document No. : ILBS#NLDB:H.9
Version No. : 1.0
Effective Date : 01/01/2025

Address
National Liver Disease Biobank,
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MATERIALS HANDLING AND DOCUMENTATION

Solid Tissue - Tissue Collection and Transportation

Number	Effective date	Pages	Author	Authorized by
ILBS#NLDB:H.9	01/01/2025	9	Mr. Satish Kumar	Dr.Chhagan Bihari
Version	**Review period**	**No. of copies**	**Approved by**	**Date**
1.0	2yrs	3	Dr. Chhagan Bihari	30/12/2024

Location	Subject
Biobank Reception area Almira	Solid Tissue - Tissue Collection and Transportation
Function	**Distribution**
To give information about Solid Tissue Collection and Transportation.	➢ HOD ➢ Biobank Reception area ➢ Master files

SCOPE AND APPLICATION:

This standard operating procedure (SOP) describes how tissues should be collected and transported

RESPONSIBILITY:

All ILBS Biobank personnel involved in tissue collection shall follow this SOP. The Biobank Coordinator/Clinician obtains consent, Porter transports tissue, Pathologist performs grossing/diagnosis, and Laboratory staff handle processing and storage.

ABBREVIATIONS

Ca	Cancer
OT	Operation Theatre
CT	Chemotherapy
HT	Hormone Therapy
Post NACT	Post-Neoadjuvanvt Chemotherapy
POST RT	Post Radiotherapy
BC	Buffy Coat
PP	Plasma
NT	Normal Tissue
TT	Tumour Tissue
UIN	Unique Identification Number
Rpm	Revolution Per Minute
DNA	Deoxyribo Nucleic Acid
RNA	Ribo Nucleic Acid
MRD	Medical Record Department
EDTA	Ethylenediaminetetra acetic Acid
BRC	Bio samples Release Committee
PAC	Pre Anesthetic Check up
QC	Quality Control
bp	base pairs
DMSO	Dimethyl Sulfoxide
FCS	Fetal Calf Serum
PBMC	Peripheral Blood Mononuclear Cells
RT	Room Temperature (18°-25°C)
EDTA	Ethylene Diamine tetra acetic acid
ACD	Acid citrate dextrose

1. PURPOSE

The purpose of this document is to outline standardized procedures for ILBS Biobank to follow the process of tissue collection and transportation from the operating room to the pathology laboratory. Tissue samples are collected from patients that have given their consent to participate in the biobank program only if there is tissue in excess of that required for pathological assessment and diagnosis.

2. SCOPE

This standard operating procedure (SOP) describes how tissues should be collected and transported

3. ROLES AND RESPONSIBILITIES

The SOP applies to all personnel from ILBS Biobank responsible for collecting tissue from the consented participant.

Biobank Coordinator/Clinician: Obtain Patient Consent

Operating Room Nurse: Notifies Pathologist and Biobank prior to resection.

Laboratory Technician/Technologist: Transportation of tissue, harvesting, processing and storage.

Porter: Transport of tissue from the operating room to the grossing area.

Pathologist: Diagnosis of Tissue Malignancy, grossing of tissue and resection of excess tissue for the biobank.

4. MATERIALS, EQUIPMENT AND FORMS

- Container with ice
- Appropriate container for resected tissue
- Markers and pens
- Clean Forceps
- Cold saline for rinsing tissue if needed
- Clean Scalpels for trimming tissue
- Tissue Collection kits (Tubes containing cold culture media or Phosphate Buffered Saline)
- Gloves
- Face shield/safety goggles
- Closed toed shoes
- Appropriate labels
- Tissue Collection/Harvesting Worksheets

5. PROCEDURES

This procedure is intended to ensure that tissue samples will be collected from consented participants in a safe, timely, and efficient manner while eliminating the risks of contamination. To facilitate the use of innovative genomic and proteomic techniques, banked tissue that has been adequately processed is vital to obtaining products with high integrity and quality.

5.1 Tissue Collection – General Considerations

- The scientific utility of the data obtained from the analysis of tissues is directly related to the quality of the tissue specimen.
- Cellular and molecular integrity are most affected by factors such as specimen and tissue type, conditions of tissue hypoxia, method of preservation, conditions of storage, pre-excision hypoxia and tissue product extraction methods. The following factors must be the focus of the process to obtain and maintain tissue with suitable integrity for innovative research:
  - Minimizing the time the tissue is subjected to hypoxic conditions, as this initiates the cell death mechanisms and subsequent degradation process.
  - Use of agents or treatments to inactivate degrading enzymes for preserving nucleic acid integrity.
  - Preservation of tissue as fresh frozen, if the intended use is for nucleic acid analysis.
  - Storage of frozen tissue and products at appropriate temperatures especially if storage is for longer periods of time.
  - Avoiding contamination with surrounding histological distinct tissue or co-processed samples if the product is intended for studies involving nucleic acid amplification.
- The tissue intended for banking shall not be formalin fixed.

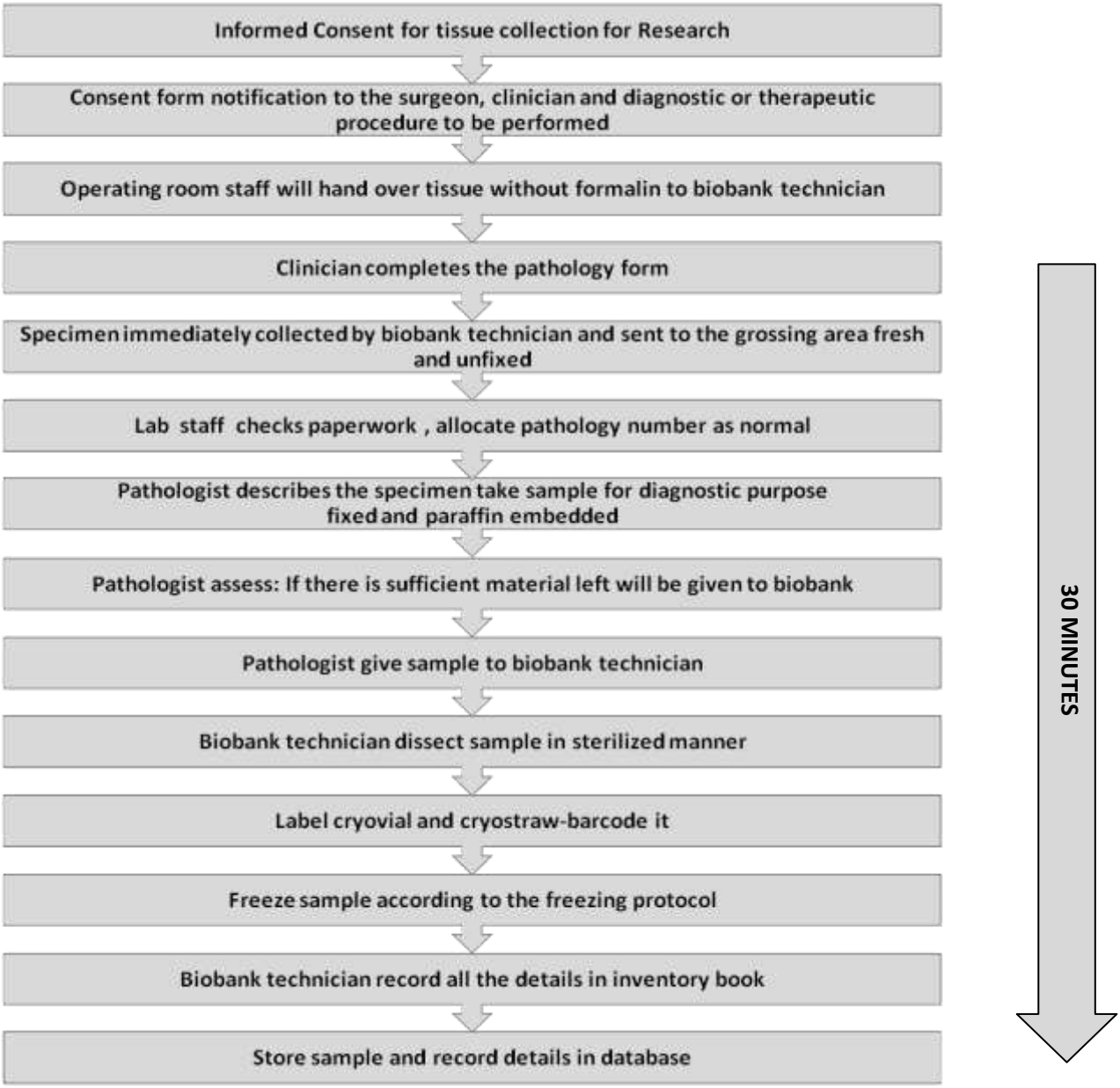
5.2 Transporting of Tissue from the Operating Room to the Pathology Laboratory

- Biobank technician will go to operating room and will wait there with all accessories and take tissue that is surplus to clinical needs and diagnosis (only tissue deemed surplus by the Pathologist is considered bankable).
- It is recommended that the operating room staff notifies about the time of ischemia (when blood vessels were clamped).
- The operating staff will hand over the fresh surgical sample without putting it into the formalin.
- Lab technician will arrange the transport or will transport the tissue from the operating room to the grossing area using a rapid specimen transport protocoloptimal for preservation of cellular and molecular integrity. It is recommended that the tissue be transported on ice.
- Pathologist will examine, note the findings and excess material which is not required/necessary for diagnosis will be given for storage.
- Biobank lab technician will harvest and store the tissue.
- Prepare tissue collection kits in advance, if possible
- No more than 30 minutes should elapse between the time of biopsy/resection and time of freezing of a given sample. Records must clearly document the actual time period.

6. APPLICABLE REFERENCES, REGULATIONS AND GUIDELINES

- 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
- US National Biospecimen Network Blueprint http://biospecimens.cancer.gov/resources/publications/reports/nbn.asp
- Human Tissue and Biological Samples for use in Research. Operational and Ethical Guidelines. Medical Research Council Ethics http://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC002420
- Jewell, S. et al. Analysis of the Molecular Quality of Human Tissues, an experience from theCooperative Human Tissue Network. Am. J. Clin. Pathol. 2002:118:733-741.

Flowchart of sample collection



TISSUE COLLECTION HARVESTING WORKSHEET

Tissue Collection

Collection Site	
Date Tissue Resection	
Time Tissue Resection	
Date Sample Received by Pathology Laboratory	
Time Sample is Received by Pathology Laboratory	
Name of Person Transporting Tissue	
Was sample transported on ice?	
Pathologist (Name)	
Additional Collection Notes	

Sample (Tissue) Information

Tissue Label (Unique identifier)	Tissue Type	Normal available and taken	Tissue Observations Noted

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Standard Operating Procedure

DOCUMENT NAME: MATERIALS HANDLING AND DOCUMENTATION

DOCUMENT NO. :ILBS#NLDB:H

Document Name : Tissue harvesting
Document No. : ILBS#NLDB:H.10
Version No. : 1.0
Effective Date : 01/01/2025

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MATERIALS HANDLING AND DOCUMENTATION

Tissue harvesting

Number	Effective date	Pages	Author	Authorized by
ILBS#NLDB:H.10	01/01/2025	11	Mr. Satish Kumar	Dr.Chhagan Bihari
Version	**Review period**	**No. of copies**	**Approved by**	**Date**
1.0	2yrs	3	Dr. Chhagan Bihari	30/12/2024

Location	Subject
Biobank Reception area Almira	Tissue harvesting
Function	**Distribution**
To give information about Tissue harvesting.	➢ HOD ➢ Biobank Reception area ➢ Master files

SCOPE AND APPLICATION:

This SOP describes the standardized procedure for harvesting tissue samples for biobanking purposes.

RESPONSIBILITY:

NLDB Biobank personnel involved in tissue collection from consented participants shall follow this SOP. The Pathologist performs diagnosis, grossing, and resection; the histopatholgy Technician assists in harvesting and transport; and the Laboratory Technician manages transport, processing, and storage.

ABBREVIATIONS

Ca	Cancer
OT	Operation Theatre
CT	Chemotherapy
HT	Hormone Therapy
Post NACT	Post-Neoadjuvanvt Chemotherapy
POST RT	Post Radiotherapy
BC	Buffy Coat
PP	Plasma
NT	Normal Tissue
TT	Tumour Tissue
UIN	Unique Identification Number
Rpm	Revolution Per Minute
DNA	Deoxyribo Nucleic Acid
RNA	Ribo Nucleic Acid
MRD	Medical Record Department
EDTA	Ethylenediaminetetra acetic Acid
BRC	Bio samples Release Committee
PAC	Pre Anesthetic Check up
QC	Quality Control
bp	base pairs
DMSO	Dimethyl Sulfoxide
FCS	Fetal Calf Serum
PBMC	Peripheral Blood Mononuclear Cells
RT	Room Temperature (18°-25°C)
EDTA	Ethylene Diamine tetra acetic acid
ACD	Acid citrate dextrose

Harvested by	
Time Frozen	
Time stored in Transporter	

Indicate if Tissue was taken for

1. Fresh Frozen Collection

Label (Identifier)	Snap Frozen by	Date Frozen	Time Frozen	Sample Size	Storagelocation

2. Frozen in OCT

Label (Identifier)	Snap Frozen by	Date Frozen	Time Frozen	Sample Size	Storagelocation

3. Formalin Fixed

Label (Identifier)		Date	Time	Sample Size	Storage location

4. Formalin Fixed

Label (Identifier)		Date	Time	Sample Size	Storage location

5. Stored in another form

Label (Identifier)		Date	Time	Sample Size	Storage location

Tissue Harvesting

1.0 PURPOSE

Tissue samples are collected from patients/donors that have been through the informed consent process and agreed to participate in the NLDB biobank program. Tissues are obtained by personnel qualified by training to do so and are collected for the NLDB biobank only if present in excess to that required for pathological assessment and diagnosis. The purpose of this document is to outline standardized procedures for NLDB biobank to follow during tissue harvesting.

2.0 SCOPE

This standard operating procedure (SOP) describes how tissues should be harvested. The SOP does not cover detailed safety procedures for handling Human Biological Materials (HBMs) and it is recommended that personnel follow institutional bio safety guidelines.

3.0 ROLES AND RESPONSIBILITIES

The SOP applies to all personnel from NLDB biobank that are responsible for collecting tissue from the consented participant. Applicable staff may include the following roles:

Bio Bank Personnel	Responsibility / Role
Pathologist	Diagnosis of Surgical/Biopsy tissue, grossing of tissue and resection of excess tissue for the biobank.
Technician	Assists with resection, harvesting and transportation of tissue and performs tasks delegated by the pathologist. May communicate with Laboratory Technician/Technologist.
Laboratory Technician/Technologist	Transportation of tissue, harvesting, processing and storage.

4.0 MATERIALS, EQUIPMENT AND FORMS

The materials, equipment and forms listed in the following list are recommendations only and may be substituted by alternative/equivalent products more suitable for the site-specific task or procedure.

Materials and Equipment	
Container with ice	
Appropriate container for resected tissue (Petri Dishes)	
Markers and pens	
Clean Forceps	
Cold Saline for rinsing tissue if needed	
Clean Scalpels for trimming tissue	
Tissue Collection kits (containing collection media if relevant)	
Gloves worn to protect personnel handling tissue	
Sufficient appropriate labels for collection tubes and Tissue Collection/Processing Worksheets	
Tissue Collection/Harvesting Worksheets	
Cryovials for storage of frozen tissue	
Dry shipper for transportation of Liquid nitrogen	
Needle/sharps disposal unit	
Optimal Cutting Temperature Compound (OCT)	

5.0 PROCEDURES

This procedure is intended to ensure that tissue samples will be collected from consented participants in a safe, timely, and efficient manner while eliminating the risks of contamination. To facilitate the use of innovative genomic and proteomic techniques, banked tissue that has been adequately processed is vital to obtaining products with high integrity and quality.

5.1 Tissue Harvesting

5.1.1 Treat all tissue as potentially infectious.

5.1.2 The pathologist performs processing unless the responsibility is delegated by the pathologist to the pathology assistant or designated NLDB biobank technician/technologist.

5.1.3 Ensure that the resected tissue never desiccates or is contaminated by surrounding tissue or other samples. If appropriate, change scalpel blades between dissecting tissue and surrounding uninvolved tissue.

5.1.4 Based on consultation with the pathologist, mark the margins with ink.

5.1.5 Slice the tissue with a clean scalpel. Always use a clean scalpel between tissue samples or between normal and tissue.

5.1.6 Select tissue for banking without compromising the tissue for pathological examination.

5.1.7 Attempt to preserve and store normal (matching) adjacent tissue as well.

5.1.8 If possible, allow for the banking of multiple samples from one specimen. The tissue may be banked as:

a. Samples snap frozen in liquid nitrogen suitable for extraction of DNA, RNA and protein.

b. Tissue samples processed directly for the extraction of DNA, RNA and protein.

c. Sample frozen in optimal cutting temperature (OCT) compound is suitable for producing frozen tissue sections.

d. Samples fixed in formalin and paraffin embedded for paraffin sections.

5.1.9 For the snap frozen tissue samples, attempt to have as many cryovials as possible.

5.1.10 Based on the tissue harvested, label the necessary cryovials, RNA or DNA tubes, cassettes for OCT or tubes for formalin processing.

5.1.11 Use cryovials suitable for submersion in liquid nitrogen.

5.1.12 It is recommended to have no less than 250 mg of tissue per vial.

5.1.13 For a small tumour/tissue attempt to harvest samples that are 2-3 mm3 (depending on /tumour tissue size and availability).

5.1.14 If there is abundant tissue, attempt to harvest about 3-4 mm3 or more (depending on size and availability).

5.1.15 Depending on the method of processing/storage, transfer the tissue to the appropriate receptacle for the processing step.

5.1.16 Timing is critical. Ideally, no more than 30 minutes must elapse between the time of biopsy/resection and time of freezing of a given sample. Records must clearly document actual time period (in hours or minutes).

SAMPLE FORM - TISSUE COLLECTION HARVESTING WORKSHEET

Tissue harvesting

The Tissue Collection/Harvesting Worksheet can be customized by specific sites to capture information relevant to the site. The following may be used as a guide for relevant sets of information to record:

Tissue Collection and Transportation

Collection Site				
Date Tissue is resected				
Time Tissue is resected				
Date Tissue Sample Received by Pathology Laboratory				
Time Sample is Received by Pathology Laboratory				
Name of Person Transporting Tissue				
Was sample transported on ice?	YES		NO	
Pathologist (Name)				
Additional Collection Notes:				

Sample Information

Label (Unique identifier)	Tissue type	Was matching normal available and taken?	Tissue size	Tissue Observations

Tissue Harvesting

Harvested by: Laboratory Technician/Technologist name

Time Frozen: Very Important to record this time

Indicate if Tissue was taken for:

1. **Fresh Frozen Collection**

Label (Identifier)	Snap Frozen by	Date Frozen	Time Frozen	Sample Size	Storage location

2. **Frozen in OCT**

Label (Identifier)	Snap Frozen by	Date Frozen	Time Frozen	Sample Size	Storage location

3. **Formalin Fixed. Yes No**

   Date: Storage Location:

4. **Stored in another form: Yes No**

   Date: Storage Location:

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Standard Operating Procedure

DOCUMENT NAME: MATERIALS HANDLING AND DOCUMENTATION

DOCUMENT NO. :ILBS#NLDB:H

Document Name : Snap Freezing of Tissue
Document No. : ILBS#NLDB:H.11
Version No. : 1.0
Effective Date : 01/01/2025

Address
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MATERIALS HANDLING AND DOCUMENTATION

Snap Freezing of Tissue

Number	Effective date	Pages	Author	Authorized by
ILBS#NLDB:H.11	01/01/2025	10	Mr. Satish Kumar	Dr.Chhagan Bihari
Version	**Review period**	**No. of copies**	**Approved by**	**Date**
1.0	2yrs	3	Dr. Chhagan Bihari	30/12/2024

Location	Subject
Biobank Reception area Almira	Snap Freezing of Tissue
Function	**Distribution**
To give information about the standardized procedure for snap freezing of tissue samples to preserve molecular integrity and ensure suitability for future analysis.	➢ HOD ➢ Biobank Reception area ➢ Master files

SCOPE AND APPLICATION:

This SOP applies to the snap freezing of tissue samples intended for preservation in biobanking settings to maintain molecular integrity for future analysis.

RESPONSIBILITY:

All personnel from NLDB biobank are responsible for snap freezing of the harvested tissue.

ABBREVIATIONS

Ca	Cancer
OT	Operation Theatre
CT	Chemotherapy
HT	Hormone Therapy
Post NACT	Post-Neoadjuvanvt Chemotherapy
POST RT	Post Radiotherapy
BC	Buffy Coat
PP	Plasma
NT	Normal Tissue
TT	Tumour Tissue
UIN	Unique Identification Number
Rpm	Revolution Per Minute
DNA	Deoxyribo Nucleic Acid
RNA	Ribo Nucleic Acid
MRD	Medical Record Department
EDTA	Ethylenediaminetetra acetic Acid
BRC	Bio samples Release Committee
PAC	Pre Anesthetic Check up
QC	Quality Control
bp	base pairs
DMSO	Dimethyl Sulfoxide
FCS	Fetal Calf Serum
PBMC	Peripheral Blood Mononuclear Cells
RT	Room Temperature (18°-25°C)
EDTA	Ethylene Diamine tetra acetic acid
ACD	Acid citrate dextrose

1.0 PURPOSE

Tissue samples (surplus to the needs of pathology) are collected from patients/donors that have been through the informed consent process and agreed to participate in the NLDB biobank program. Fresh frozen tissue collections are a valuable resource for research purposes. Tissues are only suitable for proteomic and genomic studies if frozen in a timely and appropriate manner. The purpose of this document is to outline standardized procedures for NLDB biobank to follow during snap freezing tissue. Similar procedures may be adopted for other tissues of interest that may be harvested for the biobank during surgery (such as non-malignant tissue, adjacent non-malignant tissue, lymph nodes, muscle samples, etc.); document clearly if the procedures deviate for the non- malignant samples.

2.0 SCOPE

This standard operating procedure (SOP) describes how tissues are snap frozen. The SOP does not cover detailed safety procedures for handling Human Biological Materials (HBMs) or hazardous chemicals and it is recommended that personnel follow institutional safety guidelines.

3.0 ROLES AND RESPONSIBILITIES

The policy applies to all personnel from NLDB biobank that are responsible for snap freezing of the harvested tissue.

Bio Bank Personnel	Responsibility / Role
Pathology Assistant	Assists with harvesting and transportation of tissue and performs tasks delegated by the pathologist. May communicate with Laboratory Technician/Technologist.
Laboratory Technician/Technologist	Transportation of tissue, harvesting, processing and storage.

4.0 MATERIALS, EQUIPMENT AND FORMS

The materials, equipment and forms listed in the following list are recommendations only and may be substituted by alternative/equivalent products more suitable for the site-specific task or procedure.

Materials and Equipment	
Container with dry ice (for transport of	

frozen tissue)	
Markers and pens	
Clean Forceps	
Clean Scalpels for trimming tissue	
Liquid Nitrogen	
2-Methylbutane (isopentane) (optional)	
Container for Isopentane (optional)	
Labeled cryovials for storage of frozen tissue (screw top)	
Sufficient appropriate labels for cryovials	
Dry shipper or Dewar for transportation of Liquid nitrogen	
Needle/sharps disposal unit	
Gloves worn to protect personnel handling Tissue	
Safety glasses for personnel handling liquid nitrogen tank and storage container	
Insulated gloves suitable for handling liquid nitrogen tank and storage container	
Clean underpads for bench surface	
Tissue Collection/Harvesting Worksheets Later in the protocol use harvesting worksheet	

5.0 PROCEDURES

This procedure is intended to ensure that tissue samples collected from consented participants will be frozen in a safe and efficient manner while eliminating the risks of contamination and loss of molecular integrity. To facilitate the use of genomic and proteomic techniques, banked tissue that has been adequately frozen is vital to obtaining products with high integrity and quality.

5.1 Snap Freezing of Tissue

5.1.1 Treat all tissue as potentially infectious.

5.1.2 Freezing is performed by the laboratory technician/technologist or trained personnel designated by the NLDB biobank.

5.1.3 Have materials and equipment for ready. Have as many cryovials as needed labelled and ready.

5.1.4 Unless intended for another method of preservation fresh tissue should be frozen as soon as possible. Optimally, tissue should be frozen within 30 minutes from resection.

5.1.5 Do not freeze the tissue directly on ice.

5.1.6 Ensure that the resected tissue never desiccates or is contaminated by surrounding tissue or other samples. Use clean scalpels and forceps between samples to avoid cross contamination between samples or between tumour and normal tissue.

5.1.7 Snap frozen tissue is suitable for preparation of DNA, RNA and protein. Do not place the sample in contact with formalin at any point in the process. Do not add serum to the sample.

Choose either Step 5.1.8 to 5.1.10 (snap-freezing with isopentane), or Step 5.1.11 (snap freezing with liquid nitrogen):

5.1.8 With clean forceps, place the specimen to be frozen into an empty screw capped cryovial. Close the cryovial.

5.1.9 Place the cryovial with the specimen into the container of cooled isopentane. The specimen should freeze within 30 seconds.

Or

5.1.10 Place the tissue specimen into an empty cryovial, close the cryovial, and immediately submerge the cryovial into liquid nitrogen. The specimen should freeze within 30-60 seconds. This is not recommended if the sample is large in size, as longer freezing time will result in ruined morphology.

Then

5.1.11 Once snap frozen, transfer the sample to liquid nitrogen storage container (preferred) or to an -80º C (or colder) freezer.

5.1.12 Samples should be placed on dry ice to be carried to the freezer or liquid nitrogen storage facility.

5.1.13 If storing the samples in liquid nitrogen, it is recommended that the samples be placed in the vapour phase of liquid nitrogen. Note: the glass transition temperature of water is -134^{o} C.

As such, samples intended for indefinite long-term storage should be kept at temperatures lower than -135^{o} C.

5.1.14 Record the storage location.

5.1.15 Record time of freezing on the Tissue Collection/Harvesting Worksheet. Determine time elapsed between resection and freezing and record this as well. At the very least, record the approximate time (using 15 minute increments) after resection that the tissue was frozen (i.e. Within 30 minutes or between 30-45 minutes etc).

SAMPLE FORM - TISSUE COLLECTION HARVESTING WORKSHEET

The Tissue Collection/Harvesting Worksheet can be customized by specific sites to capture information relevant to the site. The following may be used as a guide for relevant sets of information to record:

Tissue Collection and Transportation

Collection Site				
Date Tissue is resected				
Time Tissue is resected				
Date Tissue Sample Received by Pathology Laboratory				
Time Sample is Received by Pathology Laboratory				
Name of Person Transporting Tissue				
Was sample transported on ice?	YES		NO	
Pathologist (Name)				

Additional Collection Notes:	

Sample Information

Label (Unique identifier)	Tissue type	Was matching normal available and taken?	Tissue size	Tissue Observations

Tissue Harvesting

Harvested by: Laboratory Technician/Technologist name

Time Frozen: Very Important to record this time

Indicate if Tissue was taken for:

1. **Fresh Frozen Collection**

Label (Identifier)	Snap Frozen by	Date Frozen	Time Frozen	Sample Size	Storage location

2. **Frozen in OCT**

Label (Identifier)	Snap Frozen by	Date Frozen	Time Frozen	Sample Size	Storage location

3. **Formalin Fixed. Yes No**

Date: Storage Location:

4. **Stored in another form: Yes No**

Date: Storage Location:

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Standard Operating Procedure

DOCUMENT NAME: MATERIALS HANDLING AND DOCUMENTATION

DOCUMENT NO. :ILBS#NLDB:H

MATERIALS HANDLING AND DOCUMENTATION

Freezing of Tissue in Optimal Cutting Temperature (OCT)

Document Name :Freezing of Tissue in Optimal Cutting Temperature (OCT)
Document No. : ILBS#NLDB:H.12
Version No. : 1.0
Effective Date : 01/01/2025

Address
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MATERIALS HANDLING AND DOCUMENTATION

Freezing of Tissue in Optimal Cutting Temperature (OCT)

Number	Effective date	Pages	Author	Authorized by
ILBS#NLDB:H.12	01/01/2025	10	Mr. Satish Kumar	Dr.Chhagan Bihari
Version	**Review period**	**No. of copies**	**Approved by**	**Date**
1.0	2yrs	3	Dr. Chhagan Bihari	30/12/2024

Location	Subject
Biobank Reception area Almira	Freezing of Tissue in Optimal Cutting Temperature (OCT)
Function	**Distribution**
To give information about material handling and their proper documentation.	➢ HOD ➢ Biobank Reception area ➢ Master files

SCOPE AND APPLICATION:

This SOP describes the procedure for freezing tissue samples in Optimal Cutting Temperature (OCT) medium for storage and further analysis.

RESPONSIBILITY:

This SOP applies to NLDB biobank personnel involved in snap freezing of harvested tissue. Pathology Assistants assist in tissue resection and harvesting while Laboratory Technicians handle transport, processing, and storage.

ABBREVIATIONS

Ca	Cancer
OT	Operation Theatre
CT	Chemotherapy
HT	Hormone Therapy
Post NACT	Post-Neoadjuvanvt Chemotherapy
POST RT	Post Radiotherapy
BC	Buffy Coat
PP	Plasma
NT	Normal Tissue
TT	Tumour Tissue
UIN	Unique Identification Number
Rpm	Revolution Per Minute
DNA	Deoxyribo Nucleic Acid
RNA	Ribo Nucleic Acid
MRD	Medical Record Department
EDTA	Ethylenediaminetetra acetic Acid
BRC	Bio samples Release Committee
PAC	Pre Anesthetic Check up
QC	Quality Control
bp	base pairs
DMSO	Dimethyl Sulfoxide
FCS	Fetal Calf Serum
PBMC	Peripheral Blood Mononuclear Cells
RT	Room Temperature (18°-25°C)
EDTA	Ethylene Diamine tetra acetic acid
ACD	Acid citrate dextrose

MATERIALS HANDLING AND DOCUMENTATION

Freezing of Tissue in Optimal Cutting Temperature (OCT)

1.0 PURPOSE

Tissue samples (surplus to the needs of pathology) are collected from patients/donors that have been through the informed consent process and agreed to participate in the NLDB biobank program. Fresh frozen tissue collections are a valuable resource for research purposes. Tissues are only suitable for proteomic and genomic studies if cryopreserved in a timely and appropriate manner. Optimal cutting temperature (OCT) is a compound especially good for preserving cellular ultrastructure (histology) and likely nucleic acids as well. It is also used for specialized applications where formalin fixed tissue is not optimal.

2.0 SCOPE

This standard operating procedure (SOP) describes how tissues are frozen in OCT. The SOP does not cover detailed safety procedures for handling Human Biological Materials (HBMs) or hazardous chemicals and it is recommended that personnel follow institutional safety guidelines.

3.0 ROLES AND RESPONSIBILITIES

The policy applies to all personnel from NLDB biobank that are responsible for snap freezing of the harvested tissue.

Bio Bank Personnel	**Responsibility / Role**
Pathology Assistant/Technician	Assists with resection, harvesting and transportation of tissue. May communicate with Laboratory Technician/Technologist.
Laboratory Technician/Technologist	Transportation of tissue, harvesting, processing and storage.

4.0 MATERIALS, EQUIPMENT AND FORMS

The materials, equipment and forms listed in the following list are recommendations only and may be substituted by alternative/equivalent products more suitable for the site-specific task or procedure.

Materials and Equipment	
Container with dry ice (for transport of frozen tissue)	
Markers and pens	
Clean Forceps	
Clean Scalpels for trimming tissue	

Liquid Nitrogen	
2-Methylbutane (isopentane)	
Container for Isopentane	
Labeledcryovials for storage of frozen tissue (screw top)	
Plastic cryomolds	
Labeled small zip lock bags for storage of cryomolds	
Sufficient appropriate labels for cryovials, moulds and plastic bags	
Storage containers for cryomolds	
Dry shipper or Dewar for transportation of Liquid nitrogen	
Needle/sharps disposal unit	
Gloves worn to protect personnel handling Tissue	
Safety glasses for personnel handling liquid nitrogen tank and storage container	
Insulated gloves suitable for handling liquid nitrogen tank and storage container	
Clean underpads for bench surface	
Tissue Collection/Harvesting Worksheets Later in the protocol use harvesting worksheet	
Optimal Cutting Temperature Compound (OCT)	

5.0 PROCEDURES

This procedure is intended to ensure that tissue samples collected from consented participants will be frozen in a safe and efficient manner while eliminating the risks of contamination and loss of molecular integrity. To facilitate the use of genomic and proteomic techniques, banked tissue that has been adequately frozen is vital to obtaining products with high integrity and quality.

5.1 Freezing of Tissue in OCT

5.1.1 Treat all tissue as potentially infectious.

5.1.2 Freezing is performed by the laboratory technician/technologist or trained personnel designated by the NLDB biobank.

5.1.3 Have materials and equipment ready. Have as many cryovials or cryomolds as needed labelled and ready.

5.1.4 Unless intended for another method of preservation fresh tissue should be frozen as soon as possible. Optimally, tissue should be frozen within 30 minutes after resection.

5.1.5 Do not freeze the tissue directly on ice.

5.1.6 Ensure that the resected tissue never desiccates or is contaminated by surrounding tissue or other samples. Use clean scalpels and forceps between samples to avoid cross contamination between samples or between tumour and normal tissue.

5.1.7 OCT frozen tissue is suitable for preparation of DNA and RNA. It is especially useful for preserving fresh tissue intended for histopathology where morphological information is important. Do not place the sample in contact with formalin at any point in the process. Do not add serum to the sample.

5.1.8 Cool isopentane by suspending the container of isopentane in liquid nitrogen. Isopentane is sufficiently cooled when “pearls” form and the solution becomes hazy.

5.1.9 Place a few drops of the OCT compound into a pre-labelled plastic cryomolds.

5.1.10 With clean forceps, place the specimen to be frozen onto the OCT in the cryomolds. If relevant to your type of tissue, orient the tissue in the cryomolds.

5.1.11 Add more OCT to cover the tissue and fill the mould.

5.1.12 Use a forceps or transfer pipette to orient tissue and remove air bubbles.

5.1.13 Avoid introducing any air bubbles into the OCT. Release any bubbles that may become trapped around the tissue.

5.1.14 Place the cryomolds in a small container containing pre-cooled isopentane.

5.1.15 Submerge the mould in the isopentane until the OCT is completely frozen (white and solid).

5.1.16 Alternatively, the cryomolds or cryovial containing the tissue and OCT can be frozen directly in liquid nitrogen without the isopentane step. Hold the vial or cryomolds with a forceps and gently immerse the mould in liquid nitrogen contained in a dry shipper allowing for freezing to proceed from the bottom of the cryomolds or vial.

5.1.17 Remove the mould from the liquid nitrogen.

5.1.18 Place the mould into a small, labelled zip-lock bag and place the sealed bag on dry ice.

5.1.19 Transport the bags or vials on dry ice and for storage at -80º C or colder.

5.1.20 Record the storage location.

5.1.21 Record time of freezing on the Tissue Collection/Harvesting Worksheet Determine time elapsed between resection and freezing and record this as well. At the very least, record the approximate time (using 15 minute increments) after resection that the tissue was frozen (i.e. Within 30 minutes or between 30-45 minutes etc.).

SAMPLE FORM - TISSUE COLLECTION HARVESTING WORKSHEET

The Tissue Collection/Harvesting Worksheet can be customized by specific sites to capture information relevant to the site. The following may be used as a guide for relevant sets of information to record:

Tissue Collection and Transportation

MATERIALS HANDLING AND DOCUMENTATION

Freezing of Tissue in Optimal Cutting Temperature (OCT)

Collection Site				
Date Tissue is resected				
Time Tissue is resected				
Date Tissue Sample Received by Pathology Laboratory				
Time Sample is Received by Pathology Laboratory				
Name of Person Transporting Tissue				
Was sample transported on ice?	YES		NO	
Pathologist (Name)				
Additional Collection Notes:				

Sample Information

Label (Unique identifier)	Tissue type	Was matching normal available and taken?	Tissue size	Tissue Observations

Tissue Harvesting

Harvested by: Laboratory Technician/Technologist name

Time Frozen: Very Important to record this time

Indicate if Tissue was taken for:

1. **Fresh Frozen Collection**

Label (Identifier)	Snap Frozen by	Date Frozen	Time Frozen	Sample Size	Storage location

2. **Frozen in OCT**

Label (Identifier)	Snap Frozen by	Date Frozen	Time Frozen	Sample Size	Storage location

3. **Formalin Fixed. Yes No**

   Date: Storage Location:

4. **Stored in another form: Yes No**

   Date: Storage Location:

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Standard Operating Procedure

DOCUMENT NAME: MATERIALS HANDLING AND DOCUMENTATION

DOCUMENT NO. :ILBS#NLDB:H

MATERIALS HANDLING AND DOCUMENTATION

Preservation of Tissue: Paraffin Embedding

Document Name	**:**	**Preservation of Tissue: Paraffin Embedding**
Document No.	**:**	**ILBS#NLDB:H.13**
Version No.	**:**	**1.0**
Effective Date	**:**	**01/01/2025**

Address

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MATERIALS HANDLING AND DOCUMENTATION

Preservation of Tissue: Paraffin Embedding

ILBS#NLDB:H.13	01/01/2025	10	Mr. Satish Kumar	Dr.Chhagan Bihari
Version	**Review period**	**No. of copies**	**Approved by**	**Date**
1.0	2yrs	3	Dr. Chhagan Bihari	30/12/2024

Location	**Subject**
Biobank Reception area Almira	Preservation of Tissue: Paraffin Embedding
Function	**Distribution**
To give information about the standardized procedure for paraffin embedding of tissue specimens to preserve tissue morphology and integrity for storage and further analysis.	➢ HOD ➢ Biobank Reception area ➢ Master files

SCOPE AND APPLICATION:

This SOP applies to all personnel involved in the paraffin embedding of tissue specimens processed in the biobank. It covers the procedure for embedding tissues in paraffin blocks to preserve tissue morphology and facilitate storage, sectioning, and further analysis.

RESPONSIBILITY:

It is the responsibility of trained laboratory/biobank personnel to perform tissue paraffin embedding, labeling, handling, and documentation according to the established procedure.

ABBREVIATIONS

Ca	Cancer
OT	Operation Theatre
CT	Chemotherapy
HT	Hormone Therapy
Post NACT	Post-Neoadjuvanvt Chemotherapy
POST RT	Post Radiotherapy
BC	Buffy Coat
PP	Plasma
NT	Normal Tissue
TT	Tumour Tissue
UIN	Unique Identification Number
Rpm	Revolution Per Minute
DNA	Deoxyribo Nucleic Acid
RNA	Ribo Nucleic Acid
MRD	Medical Record Department
EDTA	Ethylenediaminetetra acetic Acid
BRC	Bio samples Release Committee
PAC	Pre Anesthetic Check up
QC	Quality Control
bp	base pairs
DMSO	Dimethyl Sulfoxide
FCS	Fetal Calf Serum
PBMC	Peripheral Blood Mononuclear Cells
RT	Room Temperature (18°-25°C)
EDTA	Ethylene Diamine tetra acetic acid
ACD	Acid citrate dextrose

MATERIALS HANDLING AND DOCUMENTATION

Preservation of Tissue: Paraffin Embedding

1.0 PURPOSE

Tissues are only suitable for specific research studies if preserved appropriately. To date, formaldehyde is the most widely used universal fixative because it preserves a wide range of tissues and tissue components. Formaldehyde fixed and paraffin embedded (FFPE) tissue can easily be stored under normal laboratory conditions for a long period. The method is effective for preserving histological morphology of the tissue specimen. The purpose of this document is to outline standardized procedures for NLDB biobank to follow when preserving tissue by the FFPE method.

2.0 SCOPE

This standard operating procedure (SOP) describes how tissues should be preserved by FFPE. The SOP does not cover detailed safety procedures for handling Human Biological Materials (HBMs) or hazardous chemicals and it is recommended that personnel follow institutional safety guidelines.

3.0 ROLES AND RESPONSIBILITIES

The policy applies to all personnel from NLDB biobank that are responsible for FFPE treatment of the harvested tissue.

Bio Bank Personnel	**Responsibility / Role**
Histology Laboratory Technician /Technologist	May be specifically responsible for processing FFPE tissues
Laboratory Technician/Technologist	Transportation of tissue, harvesting, processing and storage.

4.0 MATERIALS, EQUIPMENT AND FORMS

The materials, reagents, equipment and forms listed in the following list are recommendations only and may be substituted by alternative/equivalent products more suitable for the site-specific task or procedure.

Materials and Equipment	
Markers and pens	
Clean Forceps	
Clean Scalpels for trimming tissue	
Containers for fixing tissue	
Sufficient appropriate labels for tubes and	

histology cassettes.	
Histology cassettes.	
Needle/sharps disposal unit	
Gloves worn to protect personnel handling Tissue	
Clean underpads for bench surface	
Tissue Collection/Harvesting Worksheets.	
Neutral pH phosphate buffered formalin	
Alcohol (absolute, 100% Anhydrous)	
Xylene	
Paraffin	

5.0 PROCEDURES

This procedure is intended to ensure that tissue samples collected from consented participants/donors will be frozen in a safe and efficient manner while eliminating the risks of contamination and loss of molecular integrity. Banked tissue that has been adequately preserved is useful for a greater variety of studies. Consistency in procedure is important for obtaining comparable and reliable test results. Formalin fixation is standard practice in most routine histopathology laboratories. The following guidelines address specific issues related to preservation of formalin-fixed specimens.

a. Tissue specimens should not be bigger than 1.5 x 1 x 0.5 cm.

b. Under-fixation is a greater risk but also avoid over fixation as it can create problems for immunohistochemical methods.

c. Fixatives such as Bouin's which contain Picric acid must be avoided as this compound interferes with subsequent polymerase chain reaction (PCR) analysis of extracted nucleic acids.

The site may have a tissue processor, which has standardized processing times. However, use the following steps as a guide.

5.1 Fixation in Formalin

5.1.1 Treat all tissue as potentially infectious.

5.1.2 Fixation is performed by the laboratory technician/technologist or trained personnel designated by the NLDB biobank.

5.1.3 Have materials and equipment ready. Have as many containers, cassettes or vials as needed labelled and ready.

5.1.4 Fixation of tissue should be undertaken as soon as possible. Optimally, tissue should be fixed within 4 hours from resection.

5.1.5 Record time from resection to fixation.

5.1.6 Use 10% neutral pH phosphate buffered formalin as a fixative. It is important that the fixative is buffered to avoid the formation of formaldehyde pigment on blood rich tissues.

5.1.7 Perform fixation at room temperature (25º C).

5.1.8 The volume of the fixative should be at least 10-15 times greater than the volume of the tissue (i.e., 10-15 ml for every gram of tissue).

5.1.9 If needed, dissect the tissue before fixation to ensure adequate penetration of the fixative.

5.1.10 It is recommended that specimen thickness should be 2.5 mm or thinner to be adequately fixed. If this is not possible, do not use specimens that are over 4-5 mm in thickness.

5.1.11 Optimally, duration of fixation should be overnight to 24 hours but no more than 48 hours. The fixation time should be documented

5.2 Processing for Embedding

5.2.1 Dehydrate tissues through series of alcohols.

5.2.2 Clear tissue by treatment with xylene.

5.2.3 The following steps for dehydration and clearing can be can be used as a guide.

STEP	TIME	SOLUTION
2	30 min	ALCOHOL 70%
3	30 min	ALCOHOL 95%

4	30 min	ALCOHOL 100%
5	60 min	ALCOHOL 100%
6	60 min	ALCOHOL 100%
7	60 min	ALCOHOL 100%
8	60 min	XYLENE
9	60 min	XYLENE
10	60 min	XYLENE

5.2.4 After step 10 in the table continue with embedding in paraffin.

5.3 Embedding in Paraffin

5.3.1 Preferably, use low melt paraffin, as it will improve quality of nucleic acids.

5.3.2 Use the following steps as a guide to follow after step #10 in the table above.

STEP	TIME	Temperature ^{O}C	SOLUTION
11	60 min	58 ^{O}C	PARAFFIN
12	60 min	58 ^{O}C	PARAFFIN
13	60 min	58 ^{O}C	PARAFFIN

5.3.3 After completion of processing, the labelled cassettes are opened at the embedding centre.

5.3.4 Remove the tissue and place it in an appropriate sized heated mould.

5.3.5 Hold the tissue specimen down with a dissecting forceps while partially filling the mould with molten paraffin. Secure the tissue by quickly cooling the base of the mould.

5.3.6 Place the labels as appropriate and fill the mould to the top with paraffin.

5.3.7 Cool the blocks in a cooling area to set the paraffin for 30 minutes

5.3.8 Remove blocks from the mould.

5.3.9 The blocks are now ready to be sectioned or stored.

5.3.10 Store paraffin blocks at or below room temperature. Prevent exposure to sun or extreme temperature variance. Store blocks in moisture resistant cardboard boxes or plastic storage boxes.

5.3.11 Record storage location.

SAMPLE FORM - TISSUE COLLECTION HARVESTING WORKSHEET

The Tissue Collection/Harvesting Worksheet can be customized by specific sites to capture information relevant to the site. The following may be used as a guide for relevant sets of information to record:

Tissue Collection and Transportation

Collection Site				
Date Tissue is resected				
Time Tissue is resected				
Date Tissue Sample Received by Pathology Laboratory				
Time Sample is Received by Pathology Laboratory				
Name of Person Transporting Tissue				
Was sample transported on ice?	YES		NO	
Pathologist (Name)				
Additional Collection Notes:				

Sample Information

Label (Unique identifier)	Tissue type	Was matching normal available and taken?	Tissue size	Tissue Observations

Tissue Harvesting

Harvested by: Laboratory Technician/Technologist name

Time Frozen: Very Important to record this time

Indicate if Tissue was taken for:

1. **Fresh Frozen Collection**

Label (Identifier)	Snap Frozen by	Date Frozen	Time Frozen	Sample Size	Storage location

2. **Frozen in OCT**

Label (Identifier)	Snap Frozen by	Date Frozen	Time Frozen	Sample Size	Storage location

3. **Formalin Fixed. Yes No**

Date: Storage Location:

4. **Stored in another form: Yes No**

Date: Storage Location:

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Standard Operating Procedure

DOCUMENT NAME: MATERIALS HANDLING AND DOCUMENTATION

DOCUMENT NO. :ILBS#NLDB:H

Document Name : Sectioning of Tissue – Paraffin and OCT Embedded Tissue
Document No. : ILBS#NLDB:H.14
Version No. : 1.0
Effective Date : 01/01/2025

Address
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MATERIALS HANDLING AND DOCUMENTATION

Sectioning of Tissue – Paraffin and OCT Embedded Tissue

Number	Effective date	Pages	Author	Authorized by
ILBS#NLDB:H.14	01/01/2025	11	Mr. Satish Kumar	Dr.Chhagan Bihari
Version	**Review period**	**No. of copies**	**Approved by**	**Date**
1.0	2yrs	3	Dr. Chhagan Bihari	30/12/2024

Location	Subject
Biobank Reception area Almira	Sectioning of Tissue – Paraffin and OCT Embedded Tissue
Function	**Distribution**
To give information about Sectioning of Tissue – Paraffin and OCT Embedded Tissue.	➢ HOD ➢ Biobank Reception area ➢ Master files

SCOPE AND APPLICATION:

This standard operating procedure (SOP) describes how tissues preserved in paraffin and OCT should be sectioned. The SOP also outlines minimum assessment that should be in place to evaluate the quality and integrity of paraffin and frozen tissue sections.

RESPONSIBILITY:

The Pathologist is responsible for histopathological characterization. The Laboratory Technician processes FFPE tissues, performs sectioning of paraffin and OCT blocks, assists in quality control, and maintains records and documentation.

ABBREVIATIONS

Ca	Cancer
OT	Operation Theatre
CT	Chemotherapy
HT	Hormone Therapy
Post NACT	Post-Neoadjuvanvt Chemotherapy
POST RT	Post Radiotherapy
BC	Buffy Coat
PP	Plasma
NT	Normal Tissue
TT	Tumour Tissue
UIN	Unique Identification Number
Rpm	Revolution Per Minute
DNA	Deoxyribo Nucleic Acid
RNA	Ribo Nucleic Acid
MRD	Medical Record Department
EDTA	Ethylenediaminetetra acetic Acid
BRC	Bio samples Release Committee
PAC	Pre Anesthetic Check up
QC	Quality Control
bp	base pairs
DMSO	Dimethyl Sulfoxide
FCS	Fetal Calf Serum
PBMC	Peripheral Blood Mononuclear Cells
RT	Room Temperature (18°-25°C)
EDTA	Ethylene Diamine tetra acetic acid
ACD	Acid citrate dextrose

1.0 PURPOSE

Preserved tissues collected through the informed consent process are valuable for specific research studies. Formalin fixed and paraffin embedded (FFPE) tissue and tissue frozen in Optimal Cutting Temperature (OCT) compound can be sectioned for studies needing preservation of histomorphology of the specimen. For studies involving immunohistochemistry or *in situ* hybridization, sections of unfixed tissue frozen in OCT may be more appropriate. Some research studies also use sections to extract nucleic acids from specimens. The purpose of this document is to outline standardized procedures for NLDB biobank to follow when sectioning tissue preserved in paraffin or OCT.

In addition, quality control is fundamental to the successful operation of NLDB biobank offering tissue specimens for research purposes. NLDB biobank should be confident that they are providing tissue sections with high quality to appropriately meet the research needs of the investigators. Testing procedures should be in place to monitor and assess the quality and integrity of the sections released for prospective research studies.

2.0 SCOPE

This standard operating procedure (SOP) describes how tissues preserved in paraffin and OCT should be sectioned. The SOP also outlines minimum assessment that should be in place to evaluate the quality and integrity of paraffin and frozen tissue sections.

3.0 ROLES AND RESPONSIBILITIES

The SOP applies to all personnel from NLDB biobank that are responsible for sectioning tissue preserved in paraffin or OCT blocks.

Bio Bank Personnel	Responsibility / Role
Pathologist	Conduct histopathological characterization
Laboratory Technician/Technologist	May be specifically responsible for processing FFPE tissues and sectioning paraffin and OCT blocks. Conducts and assists with quality control. Records and documents outcomes.

4.0 MATERIALS, EQUIPMENT AND FORMS

The materials, equipment and forms listed in the following list are recommendations only and may be substituted by alternative/equivalent products more suitable for the site-specific task or procedure.

Materials and Equipment	
Solvent resistant markers, ink, pencils, and pens	
Microscope	
Microtome	
Hot water bath	
Microtome blades	
Fine tipped paint brush	
Fine tipped tissue separator	
Appropriate labels for slides	
Labeled glass slides	
Tray to hold slides	
Ice tray	
Oven	
Cryostat	
Labeled electrostatically charged slides (such as Super frost Plus)	
Container with dry ice for OCT blocks	
Film for sealing slide boxes such as Parafilm	
Slide storage boxes and/or slide shippers	
Optimal Cutting Temperature Compound (OCT)	
Harris Hematoxylin (filtered)	
Eosin	

5.0 PROCEDURES

This procedure is intended to ensure that tissue samples preserved for research studies are sectioned in a safe and consistent manner while eliminating the risks of contamination and loss of molecular and structural integrity. It also ensures rationing of the tissue blocks associated for each case for multiple assays and projects and maintenance of the block orientation. Consistency in procedure is important for obtaining comparable and reliable test results.

These procedures also outline minimum steps that should be followed to ensure that tissue samples collected stored and distributed are of sufficient morphological and molecular calibre to meet the research needs of the investigators.

5.1 Sectioning Formalin Fixed Paraffin Embedded Tissue

5.1.1 Treat all tissue as potentially infectious.

5.1.2 Sectioning is performed by the laboratory or histology technician/technologist or personnel trained to use a microtome and cut histological sections.

5.1.3 Have materials and equipment ready. Have as many slides as needed labelled and ready.

5.1.4 Pre-cool paraffin blocks, tissue side down, on a tray of ice. In some cases this may facilitate sectioning. Using a steel microtome knife or disposable blade cut sections that are 4-5 microns for histological sections, and 5-10 microns for nucleic acid extraction and up to 20 microns for protein extraction purposes.

5.1.5 For histological sections label slides serially.

5.1.6 Dry paraffin sections at 37° C overnight, although this depends on purpose.

5.1.7 Remove the sections from the oven and allow cooling at room temperature.

5.1.8 The sections are stored for shipping in slide mailers or stored in slide holder boxes most often at room temperature. Extended storage (usually more than 3 days) of unstained FFPE slides should be avoided as this may result in the loss of antigens. While not established, vacuum sealing and refrigeration may help preserve some unstable antigens.

5.1.9 For nucleic acid extraction sections, allow the individual sections to roll up naturally and place them directly into sterile microfuge tubes ready for nucleic acid extraction. The extraction buffer can be added directly to the microfuge tube in order to preserve the molecular integrity of the sample. When cutting sections for DNA or RNA extraction, all instruments and equipment must be pre-cleaned and wiped down with RNAse-away before and between each specimen. Gloves must be worn. Molecular grade water must be used for floating sections for RNA extraction. If sections require floating for RNA analysis, molecular grade water must be used.

5.2 Sectioning OCT Embedded Tissue

Sectioning tissue can be dangerous and carries a biohazard risk. Personnel sectioning tissue should receive adequate training in operating sectioning equipment and using safety precautions.

5.2.1 Frozen sections are cut by personnel specifically trained to perform the task of sectioning OCT embedded tissue in a cryostat. The frozen tissue cryomolds or vials are transferred to the cryostat on dry ice.

5.2.2 Set the section thickness at 4-5 microns for immunohistochemistry, *in situ* hybridization or Haematoxylin and Eosin and 5-10 microns for nucleic acid extraction and upto 20 microns for protein extraction samples. Since OCT may interfere with the further manipulation of nucleic acids, it is recommended that if the sections are to be extracted for nucleic acids, care should be taken to avoid OCT contamination of the sample.

5.2.3 Sections are mounted on room temperature slides by inverting the slide on a slight angle over the section as it lies on the knife back. The section will be attracted to the slide electrostatically. However, the slide should be placed at -20° C after 30 minutes at room temperature. Alternatively, the section can be fixed immediately in cold 95% ethanol immediately after electrostatic adherence to the slide and processed immediately.

5.2.4 For nucleic acid or protein extraction, simply allow the tissue sections to roll naturally and place them into pre-labelled, pre-cooled microfuge tubes. Samples can be stored at -80° C or alternatively the appropriate extraction buffer can be added immediately and samples processed or stored at -80° C.

5.2.5 When sectioning is done, remove the block carefully from the specimen disc. Then reseal the block with foil and immediately place it on dry ice for return to cryostorage. When cutting sections for DNA or RNA extraction, all instruments and equipment must be pre-cleaned and wiped down with RNase-away. Clean the cryostat mix with 70% ethanol on sterile gauze (to prevent freezing) before and between each specimen. Gloves must be worn. Molecular grade water must be used for floating sections for RNA extraction.

5.2.6 Frozen sections on slides not requiring a fixation step can go directly into pre-cooled plastic slide boxes or slide mailers sealed with Parafilm for storage in a -80° C freezer.

NOTE: During the sectioning procedure avoid allowing the OCT blocks to warm up. In particular, avoid cycles of heating and cooling.

5.3 Quality Assessment – General Considerations for Section Review

5.3.1 At a minimum, assessment must consist of morphologic review of tissue sections.

5.3.2 Use researcher feedback about section quality to refine practices and guide evolution of Quality Control procedures.

5.4 Quality Assessment – Issues Concerning Quality of Sections

5.4.1 Make sure that representative tissue remains in the block after sections are cut for an assay. Do not completely deplete paraffin or frozen blocks.

5.4.2 Make sure there is sufficient material on a histological section for the intended assay without compromising representative material in the tissue block.

5.4.3 Ensure that the block used for tissue sectioning is appropriate for the purpose of the intended assay. (e.g., for a study of invasive cancer, representative invasive cancer cells need to be present in sufficient quantity on all sections provided for the study).

5.4.4 If sections are intended for Polymerase Chain Reaction (PCR)-based molecular studies make sure that all attempts are made to eliminate or minimize nucleic acid contamination from equipment or other samples.

5.4.5 Ensure that type of fixation, processing duration and temperatures used during the fixation and sectioning procedures minimize the antigen masking or deterioration of molecular components. This is important for certain proteins in assays such as immunohistochemistry.

5.4.6 Ensure that section thickness is consistent and appropriate for intended use.

5.4.7 Ensure that sections are not scored or torn by the microtome knife as this will obscure microscopic observation and may cause uneven staining or bias assay results.

5.4.8 Ensure that thin sections are placed on electrostatically charged slides to avoid loss of the section during the assay.

5.4.9 Ensure that paraffin and frozen sections are stored and shipped under appropriate conditions and temperatures.

5.5 Quality Assessment – General Sectioning Regimen for QA Safeguards

The use of this schema is recommended to ensure that representative sections from a sectioned block are kept for quality assessment purposes. Perform these steps at the time the block is being sectioned for a research application.

5.5.1 Ensure that a representative Hematoxylin and Eosin (H&E) section is retained from the block within the biobank.

5.5.2 If no H&E is available from the last sectioning of the block retain a "top" section for H&E review.

5.5.3 If many sections are taken from a block, it may be useful to retain "intermediate" sections from the tissue block for H&E review.

5.5.4 Label sections serially. Also record the date the section is cut.

SAMPLE FORM - TISSUE COLLECTION HARVESTING WORKSHEET

The Tissue Collection/Harvesting Worksheet can be customized by specific sites to capture information relevant to the site. The following may be used as a guide for relevant sets of information to record:

Tissue Collection and Transportation

Collection Site				
Date Tissue is resected				
Time Tissue is resected				
Date Tissue Sample Received by Pathology Laboratory				
Time Sample is Received by Pathology Laboratory				
Name of Person Transporting Tissue				
Was sample transported on ice?	YES		NO	
Pathologist (Name)				

Additional Collection Notes:	

Sample Information

Label (Unique identifier)	Tissue type	Was matching normal available and taken?	Tissue size	Tissue Observations

Tissue Harvesting

Harvested by: Laboratory Technician/Technologist name

Time Frozen: Very Important to record this time

Indicate if Tissue was taken for:

1. **Fresh Frozen Collection**

Label (Identifier)	Snap Frozen by	Date Frozen	Time Frozen	Sample Size	Storage location

2. **Frozen in OCT**

Label (Identifier)	Snap Frozen by	Date Frozen	Time Frozen	Sample Size	Storage location

3. **Formalin Fixed. Yes No**

Date: Storage Location:

4. **Stored in another form: Yes No**

Date: Storage Location:

National
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Disease
Biobank

Standard Operating Procedure

DOCUMENT NAME: MATERIALS HANDLING AND DOCUMENTATION

DOCUMENT NO. :ILBS#NLDB:H

Document Name : Haematoxylin& Eosin Staining of Tissue Sections
Document No. : ILBS#NLDB:H.15
Version No. : 1.0
Effective Date : 01/01/2025

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MATERIALS HANDLING AND DOCUMENTATION

Haematoxylin& Eosin Staining of Tissue Sections

Number	Effective date	Pages	Author	Authorized by
ILBS#NLDB:H.15	01/01/2025	8	Mr. Satish Kumar	Dr.Chhagan Bihari
Version	**Review period**	**No. of copies**	**Approved by**	**Date**
1.0	2yrs	3	Dr. Chhagan Bihari	30/12/2024

Location	Subject
Biobank Reception area Almira	Haematoxylin & Eosin Staining of Tissue Sections
Function	**Distribution**
To give information about the standardized Haematoxylin & Eosin staining procedure for tissue sections for microscopic evaluation..	➢ HOD ➢ Biobank Reception area ➢ Master files

SCOPE AND APPLICATION:

This SOP describes the procedure for Haematoxylin and Eosin staining of tissue sections for microscopic examination. It applies to all routine histological processing of tissue samples within the Biobank laboratory.

RESPONSIBILITY:

Histopathology Technicians are responsible for processing FFPE and frozen (OCT) tissue samples, including sectioning and performing Haematoxylin & Eosin staining.

ABBREVIATIONS

Ca	Cancer
OT	Operation Theatre
CT	Chemotherapy
HT	Hormone Therapy
Post NACT	Post-Neoadjuvanvt Chemotherapy
POST RT	Post Radiotherapy
BC	Buffy Coat
PP	Plasma
NT	Normal Tissue
TT	Tumour Tissue
UIN	Unique Identification Number
Rpm	Revolution Per Minute
DNA	Deoxyribo Nucleic Acid
RNA	Ribo Nucleic Acid
MRD	Medical Record Department
EDTA	Ethylenediaminetetra acetic Acid
BRC	Bio samples Release Committee
PAC	Pre Anesthetic Check up
QC	Quality Control
bp	base pairs
DMSO	Dimethyl Sulfoxide
FCS	Fetal Calf Serum
PBMC	Peripheral Blood Mononuclear Cells
RT	Room Temperature (18°-25°C)
EDTA	Ethylene Diamine tetra acetic acid
ACD	Acid citrate dextrose

1.0 PURPOSE

Tissue samples are collected from patients/donors that have been through the informed consent process and agreed to participate in the NLDB biobank program. Tissues are preserved and are valuable for specific research studies. Formalin fixed and paraffin embedded (FFPE) tissue and tissue frozen in Optimal cutting temperature (OCT) compound can be sectioned for studies needing preservation of histomorphology of the specimen. Staining of the sections with Haematoxylin and Eosin (H&E) is employed universally for microscopic examination of tissue. It facilitates interpretation of pathology, identification of tissue, study of tissue composition and accurate tissue/tumour grading. If many sections are cut from a tissue block, H&E sections may have to be done at intervals to ensure representation of the tumour/tissue.

2.0 SCOPE

This standard operating procedure (SOP) describes how sections of tissues should be stained.

3.0 ROLES AND RESPONSIBILITIES

The SOP applies to all personnel from NLDB biobank that are responsible for sectioning and staining tissue preserved in paraffin or OCT blocks.

Bio Bank Personnel	Responsibility / Role
Histology Laboratory Technician / Technologist	Responsible for processing FFPE tissues, sectioning paraffin and frozen (OCT) samples and staining tissue sections.

4.0 MATERIALS, EQUIPMENT AND FORMS

The materials, equipment and forms listed in the following list are recommendations only and may be substituted by alternative/equivalent products more suitable for the site-specific task or procedure.

Materials and Equipment	
Solvent resistant markers, ink, pencils, and pens	
Eosin	
Harris Hematoxylin (filtered)	
Xylene/Toluene	
Tap water	
Ethanol	
HCl	

Coplin jars for staining	
Slide racks for staining and drying slides	
Forceps	
Mounting medium such as Permount and droppers	
Coverslips	
Eosin	

5.0 PROCEDURES

This procedure is intended to ensure that tissue sections are stained in a consistent manner. As mentioned earlier stained sections are valuable for studying tissue morphology and structure. Microscopic examination of stained sections facilitates identification of tissue and components. Consistency in procedure is important for obtaining comparable and reliable test results. Times specified for the steps in the protocol may be modified to suit laboratory specific reagents, which may vary slightly in strengths and composition.

5.1 Staining of Formalin Fixed Paraffin Embedded Tissue Sections

5.1.1 Treat all tissue as potentially infectious.

5.1.2 Staining is performed by the laboratory or histology technician/technologist or trained personnel designated by the NLDB biobank.

5.1.3 Have materials and equipment ready. Have reagents and equipment ready.

5.1.4 Take sections FFPE, sections that have been cut or slides from storage.

5.1.5 Dewaxing

REAGENT	TIME	EQUIPMENT
Xylene/Toluene	2-4 minutes with occasional agitation	Slide holder Staining dish with lids In fume hood
Xylene/Toluene	2-4 minutes with occasional agitation	Slide holder Staining dish with lids In fume hood

5.1.6 Rehydration

REAGENT	TIME	EQUIPMENT
100% Ethanol	2 minutes	Slide holder Staining dish
100% Ethanol	2 minutes	Slide holder Staining dish
85% Ethanol	2 minutes	Slide holder

		Staining dish
70% Ethanol	2 minutes	Slide holder Staining dish
Slowly running water wash	5 minutes	Slide holder

5.1.7 Staining

REAGENT	TIME
Harris Haematoxylin	4 minutes
Slowly running water wash	5 minutes
Acid- Alcohol (1% HC1 in 95% ethanol) (destain)	1-5 dips (as needed to destain to required degree)
Slowly running water wash	8 minutes
Ammonia water	2 minutes
Slowly running water wash	5 minutes
Eosin	2 minutes

5.1.8 Dehydration

REAGENT	TIME
70% Ethanol	5-10 dips
85-95% Ethanol	10 dips
100% Ethanol	2 minutes
Clear in Xylene/Toluene	2 minutes
Clear in Xylene/Toluene	2 minutes

5.1.9 Cover slip slides with mounting medium such as Permount.

5.1.10 Staining Results:

- Nuclei (Deep Blue)
- Cytoplasm and connective tissue (shades of pink)

5.2 Staining of OCT Embedded Tissue Sections

5.2.1 The main differences from the protocol described for FFPE sections above are due to the tendency for OCT embedded tissue to lift off and slide more easily during staining procedures. The use of adhesive slides may alleviate this problem. Also, Eosin staining is depressed while staining of nuclei is enhanced.

5.2.2 Staining

REAGENT	TIME
If using 95% Ethanol	1-2 minutes

70% Ethanol	1-2 minutes
Slowly running water wash	1-2 minutes
Harris Haematoxylin	30 seconds
Slowly running water wash	1-2 minutes
Acid-Alcohol (destain)	1-2 dips (or as needed to destain to required degree)*
Slowly running water wash	1-2 minutes
Ammonia water	60 seconds
Slowly running water wash	1-2 minutes
70% Ethanol	1 minute
95% Ethanol	1 minute
Eosin (1%)	30 seconds

***When dipping, do so very slowly to minimize section loss.**

5.2.3 Dehydration

REAGENT	TIME
70% Ethanol	5 dips*
85% Ethanol	10 dips*
100% Ethanol	1 minute
100% Ethanol	1 minute
Clear in Xylene/Toluene	2 minutes
Clear in Xylene/Toluene	2 minutes

***When dipping, do so very slowly to minimize section loss.**

5.2.4 Cover slip slides with mounting medium such as Permount.

5.2.5 Staining Results:

- Nuclei (Deep Blue)
- Cytoplasm and connective tissue (shades of pink)

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Standard Operating Procedure

DOCUMENT NAME: MATERIALS HANDLING AND DOCUMENTATION

DOCUMENT NO. :ILBS#NLDB:H

Document Name : **Tissue Derivatives – Extraction of DNA**
Document No. : **ILBS#NLDB:H.16**
Version No. : **1.0**
Effective Date : **01/01/2025**

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MATERIALS HANDLING AND DOCUMENTATION

Tissue Derivatives – Extraction of DNA

Number	Effective date	Pages	Author	Authorized by
ILBS#NLDB:H.16	01/01/2025	8	Mr. Satish Kumar	Dr.Chhagan Bihari
Version	**Review period**	**No. of copies**	**Approved by**	**Date**
1.0	2yrs	3	Dr. Chhagan Bihari	30/12/2024

Location	Subject
Biobank Reception area Almira	Tissue Derivatives – Extraction of DNA
Function	**Distribution**
To give information about the extraction of DNA from tissue samples for biobanking applications.	➢ HOD ➢ Biobank Reception area ➢ Master files

SCOPE AND APPLICATION:

This SOP describes the standardized procedure for extraction of DNA from tissue samples for biobanking purposes.

RESPONSIBILITY:

It is the responsibility of trained laboratory personnel to perform DNA extraction from tissue samples, ensure proper labeling and documentation, and maintain quality control.

ABBREVIATIONS

Ca	Cancer
OT	Operation Theatre
CT	Chemotherapy
HT	Hormone Therapy
Post NACT	Post-Neoadjuvanvt Chemotherapy
POST RT	Post Radiotherapy
BC	Buffy Coat
PP	Plasma
NT	Normal Tissue
TT	Tumour Tissue
UIN	Unique Identification Number
Rpm	Revolution Per Minute
DNA	Deoxyribo Nucleic Acid
RNA	Ribo Nucleic Acid
MRD	Medical Record Department
EDTA	Ethylenediaminetetra acetic Acid
BRC	Bio samples Release Committee
PAC	Pre Anesthetic Check up
QC	Quality Control
bp	base pairs
DMSO	Dimethyl Sulfoxide
FCS	Fetal Calf Serum
PBMC	Peripheral Blood Mononuclear Cells
RT	Room Temperature (18°-25°C)
EDTA	Ethylene Diamine tetra acetic acid
ACD	Acid citrate dextrose

1.0 PURPOSE

Tissue samples are collected from patients/donors that have been through the informed consent process and agreed to participate in the NLDB biobank program. Genomic studies often utilize nucleic acids (DNA and RNA) derived from these samples. When extracting and storing deoxyribonucleic acid (DNA) from tissue specimens all efforts should be made to avoid contamination, prevent degradation and preserve molecular integrity. The purpose of this document is to outline standardized procedures for NLDB biobank to follow when extracting DNA from tissue samples.

2.0 SCOPE

The standard operating procedure (SOP) describes how DNA should be extracted from tissues. The SOP does not cover detailed safety procedures for handling Human Biological Materials (HBMs) or hazardous chemicals and it is recommended that personnel follow institutional safety guidelines.

3.0 ROLES AND RESPONSIBILITIES

This procedure applies to all personnel from NLDB biobank who are responsible for extracting DNA from tissue.

Bio Bank Personnel	**Responsibility / Role**
Laboratory Technician/Technologist	Responsible for labeling tubes and extracting DNA from tissue and storing samples.

4.0 MATERIALS, EQUIPMENT AND FORMS

The materials, equipment and forms listed in the following list are recommendations only and may be substituted by alternative/equivalent products more suitable for the site-specific task or procedure.

Materials and Equipment	
Markers, ink and pens	
Appropriate labels for vials	
Microfuge tubes	
Racks for micro gauge tubes	
Hot water bath (set at 55° C)	
Tube racks for water bath	
TRIS saturated phenol	
Rolling rack (nutator mixer)	

Microcentrifuge	
Pipettors for large glass pipettes	
Glass pipettes for transferring phenols and chloroforms (do not use polystyrene)	
Micropipettors	
Sterile pipette tips	
Buffer A*	
Proteinase K*	
20% SDS*	
TRIS Buffer*	
TRIS EDTA (TE) Buffer*	
Phenol chloroform/Isoamyl alcohol*	
Chloroform /Isoamyl alcohol*	
95% Ethanol	
-80º C or -20º C freezer	

5.0 PROCEDURES

This procedure is intended to ensure that DNA is extracted from tissue samples in a safe and consistent manner while eliminating the risks of contamination and loss of molecular and structural integrity. Consistency in procedure is important for obtaining comparable and reliable test results. The following steps are based on extractions protocols for quality control conducted by NLDB.

5.1 Extraction of DNA from Tissue

NOTE: Volumes indicated are recommendations only and should be scaled according to the size of the tissue sample.

5.1.1 Treat all tissue as potentially infectious.

5.1.2 DNA Extraction is performed by the laboratory technician/technologist or trained personnel designated by the NLDB biobank.

5.1.3 Have materials and equipment ready. Have as many tubes and cryovials as needed labelled and ready.

5.1.4 Suspend frozen tissue in 500 µl of Buffer A. Cut tissue (mince) into small pieces with a sterile scissors or scalpel blade. Alternatively, frozen tissue can be wrapped in aluminium foil and fragmented with a hammer.

5.1.5 Add 20 µl of 20% SDS and 20 µl of Proteinase K (10 mg/ml).

5.1.6 Incubate 3 hours or overnight in 55º C water bath (with agitator).

5.1.7 Add 500 µl of TRIS saturated phenol.

5.1.8 Mix for 10 minutes at room temperature on a nutator.

5.1.9 Centrifuge at 18, 0000 x g for 10 min at room temperature.

5.1.10 Transfer supernatant to a clean microfuge tube.

5.1.11 Add 500 µl of phenol/chloroform/Isoamyl alcohol.

5.1.12 Mix by inverting tubes.

5.1.13 Repeat steps 5.1.9 and 5.1.10.

5.1.14 Add 500uL of chloroform/Isoamyl alcohol.

5.1.15 Mix by inverting tubes.

5.1.16 Repeat steps 5.1.9 and 5.1.10.

5.1.17 Add 1 ml cold 95% ethanol to the supernatant.

5.1.18 Incubate at -80º C for 30 minutes to 2 hours or at -20º C overnight.

5.1.19 Precipitated DNA will resemble a white gelatinous fibre; scoop this with a clean pipette tip into 500µl of 70% ethanol in a microfuge tube.

5.1.20 Centrifuge at 18, 0000 x g for 5 minutes at room temperature.

5.1.21 Remove the supernatant and let the DNA dry for 10 minutes at room temperature (or until alcohol evaporates).

5.1.22 Re-suspend the DNA pellet in TE buffer to get an appropriate concentration of DNA in solution.

5.1.23 Incubate the tube in a 55º C water bath (with agitation) for one hour to dissolve the pellet.

5.1.24 DNA can be stored at 4º C.

5.1.25 For longer term store DNA at -20º C or lower. Avoid subjecting the DNA to freeze/thaw to prevent DNA fragmentation).

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DOCUMENT NAME: MATERIALS HANDLING AND DOCUMENTATION

DOCUMENT NO. :ILBS#NLDB:H

Document Name : Tissue Derivatives – Extraction of RNA
Document No. : ILBS#NLDB:H.17
Version No. : 1.0
Effective Date : 01/01/2025

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MATERIALS HANDLING AND DOCUMENTATION

Tissue Derivatives – Extraction of RNA

Number	Effective date	Pages	Author	Authorized by
ILBS#NLDB:H.17	01/01/2025	9	Mr. Satish Kumar	Dr.Chhagan Bihari
Version	**Review period**	**No. of copies**	**Approved by**	**Date**
1.0	2yrs	3	Dr. Chhagan Bihari	30/12/2024

Location	Subject
Biobank Reception area Almira	Tissue Derivatives – Extraction of RNA
Function	**Distribution**
To give information about the extraction of RNA from tissue samples for biobanking applications	➢ HOD ➢ Biobank Reception area ➢ Master files

SCOPE AND APPLICATION:

This SOP describes the standardized procedure for extraction of RNA from tissue samples for biobanking purposes.

RESPONSIBILITY:

It is the responsibility of trained laboratory personnel to perform RNA extraction from tissue samples, ensure proper labeling and documentation, and maintain quality control.

ABBREVIATIONS

Ca	Cancer
OT	Operation Theatre
CT	Chemotherapy
HT	Hormone Therapy
Post NACT	Post-Neoadjuvanvt Chemotherapy
POST RT	Post Radiotherapy
BC	Buffy Coat
PP	Plasma
NT	Normal Tissue
TT	Tumour Tissue
UIN	Unique Identification Number
Rpm	Revolution Per Minute
DNA	Deoxyribo Nucleic Acid
RNA	Ribo Nucleic Acid
MRD	Medical Record Department
EDTA	Ethylenediaminetetra acetic Acid
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PAC	Pre Anesthetic Check up
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DMSO	Dimethyl Sulfoxide
FCS	Fetal Calf Serum
PBMC	Peripheral Blood Mononuclear Cells
RT	Room Temperature (18°-25°C)
EDTA	Ethylene Diamine tetra acetic acid
ACD	Acid citrate dextrose

1.0 PURPOSE

Tissue samples are collected from patients/donors that have been through the informed consent process and agreed to participate in the NLDB biobank program. Genomic studies often utilize nucleic acids (DNA and RNA) derived from these samples. When extracting and storing RNA from tissue specimens all efforts should be made to avoid contamination, prevent degradation and preserve molecular integrity.

2.0 SCOPE

The standard operating procedure (SOP) describes how RNA should be extracted from snap frozen tissue and tissue frozen in Optimal Cutting Temperature (OCT) compound. The SOP does not cover detailed safety procedures for handling Human Biological Materials (HBMs) or hazardous chemicals and it is recommended that personnel follow institutional safety guidelines.

3.0 ROLES AND RESPONSIBILITIES

This procedure applies to all personnel from NLDB biobank who are responsible for extracting RNA from tissue.

Bio Bank Personnel	**Responsibility / Role**
Laboratory Technician/Technologist	Responsible for labeling tubes and extracting RNA from tissue and storing samples.

4.0 MATERIALS, EQUIPMENT AND FORMS

The materials, equipment and forms listed in the following list are recommendations only and may be substituted by alternative/equivalent products more suitable for the site-specific task or procedure.

Materials and Equipment	
Markers, ink and pens	
Appropriate labels for vials and microfuge tubes	
Microfuge tubes	
Racks for micro gauge tubes and microcentifuge	
Homogenizer such as a Tissue Ruptor glass-TEFLON homogenizer	
Qiacube automatic extractor	
Qiacube special microtubes, tips and rotor adapters	
Cryotome	
Micropipettors	
Sterile, Rnase-free pipet tips	

RNeasy Mini Kit	
RNeasy Micro Kit	
14.3M ß -Mercaptoethanol (ß-Me) or 2 M dithiothreitol (DTT)	
96-100% ethanol	
Disposable gloves	
70% ethanol	
-80º C or -20º C freezer	
Ice for cooling tubes and water	
Dry ice for transporting OCT blocks or frozen tissues	

5.0 PROCEDURES

This procedure is intended to ensure that RNA is extracted from tissue samples in a safe and consistent manner while eliminating the risks of contamination and loss of molecular and structural integrity. Consistency in procedure is important for obtaining comparable and reliable test results. The following steps are based on extractions protocols for quality control conducted by NLDB.

5.1 Extraction of RNA from Frozen Tissue

NOTE: Volumes indicated are recommendations only and should be scaled according to the size of the tissue sample. Make sure that all tubes, homogenizers etc. used in the RNA extraction process are RNase free or treated with RNase inhibitors**.** This protocol relies on the RNeasy Mini kit.

5.1.1 Treat all tissue as potentially infectious.

5.1.2 RNA extraction is performed by the laboratory technician/technologist or trained personnel designated by the NLDB biobank.

5.1.3 Have materials and equipment ready. Have as many tubes and cryovials as needed labelled and ready. All equipment and reagents that come in contact with the sample should be Rnase free.

5.1.4 Homogenization. Tissue samples are kept frozen at -80º C until homogenization.

5.1.5 Homogenize tissue samples (maximum 30 mg) in 600 uL of Buffer RLT* using a glass-Teflon or power homogenizer. Alternate RNase free methods for homogenizing frozen tissue can be used if a homogenizer is not

available. (Refer to Step 3 of RNeasy Mini Handbook in the section entitled Animal Tissues).

5.1.6 Centrifuge the lysate for 3 min at full speed (>18,000 x g). Carefully remove the supernatant by pipetting, and transfer it to a new microcentrifuge tube.

5.1.7 Proceed with the RNeasy Mini Qiacube protocol, or for manual processing continue with steps 5-12 described in the RNeasy Mini Handbook.

5.1.8 Store the dissolved RNA at -80º C or lower.

5.1.9 Record the storage location.

5.2 Extraction of RNA from Tissue Frozen in OCT

NOTE: This protocol relies on the RNeasy Micro kit, which is favoured, as tissue sections tend to be small. However, for larger samples, use the RNeasy Mini kit as described above.

5.2.1 Treat all tissue as potentially infectious.

5.2.2 RNA extraction from tissue embedded in OCT is performed by the laboratory technician/technologist or trained personnel designated by the NLDB biobank.

5.2.3 Have materials and equipment ready. Have as many tubes and cryovials as needed labelled and ready. All equipment and reagents that come in contact with the sample should be RNase free.

5.2.4 Use RNeasy Micro extraction kit for RNA isolation from tissue embedded in OCT.

5.2.5 Take several (5-10) 3µm OCT sections using a cryostat and place them in a pre-cooled microfuge tube. Make sure that the sections do not thaw before the next step.

5.2.6 Add 600µl Buffer RLT * and bring to room temperature.

5.2.7 Centrifuge for 12 minutes at maximum speed (>18, 0000 x g).

5.2.8 Remove supernatant fluid, but not surface layer, into new tube. Discard the rest.

5.2.9 Add 600µl 70% ethanol, mix using a pipet.

5.2.10 Take up to 700µl of that solution and run through a supplied mini column. Centrifuge for 15 seconds at 8,000 x g.

5.2.11 Repeat the previous step until all of the solution has run through the mini column.

5.2.12 Run 700µl Buffer RW1* through the mini column, centrifuge 15 seconds at 8,000 x g.

5.2.13 Change the collection tube under the mini column.

5.2.14 Run 500µl Buffer RPE* through the column, centrifuge 15 seconds at 8,000 x g.

5.2.15 Centrifuge another 500µl Buffer RPE * through the column, but this time for 2 minutes at maximum speed.

5.2.16 Change out the collection tube, spin to ensure that the column is dry for 1+ minute(s) at maximum speed. If any fluid collects in the tube, spin for another minute or two.

5.2.17 Add 30µl RNase-free H2O directly to the filter of the column, let incubate for 5-10 minutes, and spin for 1 minute at 8000 x g.

5.2.18 Store extracted RNA as above.

*The reagents (RLT, RW1, and RPE) are all supplied in the RNeasy kits.

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DOCUMENT NAME: MATERIALS HANDLING AND DOCUMENTATION

DOCUMENT NO. :ILBS#NLDB:H

Document Name : Tissue Micro Arrays from Paraffin Embedded Blocks

Document No. : ILBS#NLDB:H.18
Version No. : 1.0
Effective Date : 01/01/2025

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MATERIALS HANDLING AND DOCUMENTATION

Tissue Micro Arrays from Paraffin Embedded Blocks

Number	Effective date	Pages	Author	Authorized by
ILBS#NLDB:H.18	01/01/2025	9	Mr. Satish Kumar	Dr.Chhagan Bihari
Version	**Review period**	**No. of copies**	**Approved by**	**Date**
1.0	2yrs	3	Dr. Chhagan Bihari	30/12/2024

Location	Subject
Biobank Reception area almira	Tissue Micro Arrays from Paraffin Embedded Blocks
Function	**Distribution**
To give information about the standardized procedure for preparation of tissue microarrays from paraffin-embedded blocks for research and analytical applications.	➢ HOD ➢ Biobank Reception area ➢ Master files

SCOPE AND APPLICATION:

This SOP describes how TMAs should be constructed from FFPE tissue blocks.

RESPONSIBILITY:

- Histology Laboratory Technician- Responsible for organizing blocks, creating a template and constructing the TMA.
- Pathologist- Reads slides and chooses sections of blocks to be scored.

ABBREVIATIONS

Ca	Cancer
OT	Operation Theatre
CT	Chemotherapy
HT	Hormone Therapy
Post NACT	Post-Neoadjuvanvt Chemotherapy
POST RT	Post Radiotherapy
BC	Buffy Coat
PP	Plasma
NT	Normal Tissue
TT	Tumour Tissue
UIN	Unique Identification Number
Rpm	Revolution Per Minute
DNA	Deoxyribo Nucleic Acid
RNA	Ribo Nucleic Acid
MRD	Medical Record Department
EDTA	Ethylenediaminetetra acetic Acid
BRC	Bio samples Release Committee
PAC	Pre Anesthetic Check up
QC	Quality Control
bp	base pairs
DMSO	Dimethyl Sulfoxide
FCS	Fetal Calf Serum
PBMC	Peripheral Blood Mononuclear Cells
RT	Room Temperature (18°-25°C)
EDTA	Ethylene Diamine tetra acetic acid
ACD	Acid citrate dextrose

1.0 PURPOSE

Formaldehyde fixed and paraffin embedded (FFPE) tissue can be sectioned for studies needing preservation of histo-morphology. Conservation of the tissue resource is important to maximize the number of studies that can be conducted. Tissue Micro Arrays (TMAs) provide a cost-effective and efficient method of conserving tissue samples. TMAs have been used for molecular and immunohistochemical studies and are a valuable tool for evaluation of patient material. The purpose of this document is to outline standardized procedures for NLDB biobank to follow when creating TMAs from paraffin embedded tissue blocks.

2.0 SCOPE

This standard operating procedure (SOP) describes how TMAs should be constructed from FFPE tissue blocks. The SOP does not cover detailed safety procedures for handling Human Biological Materials (HBMs) or hazardous chemicals and it is recommended that personnel follow institutional safety guidelines.

3.0 ROLES AND RESPONSIBILITIES

The SOP applies to all personnel from NLDB biobank who are responsible for creating TMAs from FFPE tissue blocks.

Bio Bank Personnel	**Responsibility / Role**
Laboratory technician / technologist or Histology Laboratory Technician / Technologist	Responsible for organizing blocks, creating a template and constructing the TMA.
Pathologist	Reads slides and chooses sections of blocks to be scored

4.0 MATERIALS, EQUIPMENT AND FORMS

The materials, equipment and forms listed in the following list are recommendations only and may be substituted by alternative/equivalent products more suitable for the site-specific task or procedure.

Materials and Equipment	
Markers, ink and pens	
Microtome	
Hot water bath (set at 40-45°C)	
Microtome blades	
Manual tissue arrayed	

Punches with stylets (0.6- 2 mm in diameter)	
Recipient block holder	
Donor block bridge	
Tray to hold slides	
Beecher tool set for adjusting the arrayer	
Oven (set at 50-52°C)	
Appropriate labels for slides	
Labeled electrostatically charged slides (such as Super frost+)	
Tray to hold blocks to be cored	
Tray to hold blocks that have been cored	
Slide storage boxes and/or slide shippers	

5.0 PROCEDURES

This procedure is intended to ensure that tissue samples are preserved for multiple research studies and are created and sectioned in a safe, consistent and efficient manner while eliminating the risks of contamination and loss of molecular and structural integrity. The use of TMAs provides the special advantage of potentially allowing improved standardization of testing.

Consistency in procedure is important for obtaining comparable and reliable test results.

5.1 Generation of a TMA – Collecting Blocks and Information

5.1.1 Treat all tissue as potentially infectious.

5.1.2 To eliminate wastage of a tissue resource, TMA generation is performed only by experienced laboratory or histology technicians/technologists or trained personnel designated by the NLDB biobank.

5.1.3 Have materials and equipment ready.

5.1.4 Gather H&E (Hematoxylin and Eosin) slides for all cases for the pathologist to read.

5.1.5 Determine for every block if the depth of the tissue in the block is still sufficient for use in a TMA recipient block.

5.1.6 Collect information about the case and diagnosis from the archiving database as needed for the study.

5.2 Generation of a TMA – Reviewing Blocks

5.2.1 The pathologist examines the slides/ tissue blocks and marks areas that are suitable to represent the tumour/tissue as per the basis of the research study the block is being designed for.
A fine felt-tipped waterproof marker is used for marking the slides.

5.2.2 The marked areas are matched to the corresponding paraffin blocks.

5.2.3 These same areas are then marked on the paraffin block using a medium tipped marker, taking care not to damage the surface of the block by applying excessive pressure. This marks the area where the core should be removed from the donor block.

5.3 Generation of a TMA – Creating the Template

5.3.1 Use spreadsheet software such as Microsoft Excel to map out the template of the TMA. Design map to best accommodate the variety of cases, number of samples, matching normal tissue, purpose for array etc. A standard layout for a 0.6 mm core array would be to use 10 x 6 core grid which can be repeated several times (sectors) to fit the available space in the recipient block.

5.3.2 All cases on the array should be positioned randomly to avoid bias from immunohistochemical staining artifacts and biases introduced due to prior knowledge of case parameters.

5.3.3 It is good practice to insert recognizable cores at indicator positions. For example, use Mercurochrome-stained liver tissue cores at both the beginning (1 core) and end (3 cores) of the experimental cores to secure orientation and ensure correct case identification.

5.3.4 Print the spreadsheet. This is the array map.

5.4 Generation of a TMA – Recipient Block

5.4.1 Make a large blank paraffin block (25 mm x 37 mm) using a cassette mould of 15 mm in depth or more.

5.4.2 Check the newly made block for air bubbles and ensure that the block is firmly attached to the cassette.

5.4.3 Gather all blocks to be cored and place them in ordered rows in a tray. The order of the blocks in the tray should represent the order of the cores in the TMA.

5.4.4 Using a tissue arrayer such as Beecher manual tissue arrayer, measure out and mark gently on the surface of the recipient block the four corners of the array to ensure a good fit. The edges of the array should fit at least 4mm from the edge of the recipient block.

5.4.5 Create the TMA using the Beecher manual tissue arrayer following the manufacturers' procedures.

5.4.6 As each core is placed into the recipient block the block identification number should be noted on the array map. The number must be taken directly from the FFPE block to ensure that the map is an accurate representation of the actual block and not any pre-planned array map. After an FFPE block is used, return the blocks to a box in the same order as used to generate the recipient block. This system will avoid confusion as the number of the block and the order of the block in the storage box can be used to verify position in the TMA.

5.5 Generation of a TMA – Sectioning

5.5.1 Section with a new microtome knife.

5.5.2 Cut sections at 5 µ or less (2-3 µ).

5.5.3 Float the sections in a distilled water bath. Set the temperature of the water bath to no more than 5º C below the melting temperature of the paraffin used in the construction of the array. To avoid inversion of the sections on the microscope slide ensure that the sections are floated "face-up".

5.5.4 Remove sections after 5-20 seconds in the water bath and mount on electrostatically charged slides (e.g. Super frost +). Pay careful attention to orientation of the array at this step.

5.5.5 Dry the slides overnight at room temperature and then bake the slides for 20 minutes at 50º C before moving to storage.

5.6 Storage of TMAs

5.6.1 Some antigens require more stringent protection from oxidation and may require the use of freshly cut TMA slides.

5.6.2 Keep a beaker of melted paraffin in a 60º C incubator.

5.6.3 Quickly dip the air-dried slide in the paraffin once.

5.6.4 Place the slide on a flat surface and allow to cool.

5.6.5 The slides can be stored in slide storage boxes at room temperature for extended periods of time. Limit exposure to temperature variations and moisture.

5.6.6 Non-paraffin dipped/protected slides can be kept at 4º C for up to 2 months in a standard microslide box. This is sufficient for most antigens.

5.6.7 Record storage location.

5.7 Release of TMAs

5.7.1 Note that TMAs contain human biological material and release of TMAs for research studies must be according to procedures outlined in NLDB *Material Request and Release.*

National
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Standard Operating Procedure

DOCUMENT NAME: MATERIALS HANDLING AND DOCUMENTATION

DOCUMENT NO. :ILBS#NLDB:H

Sample retrieval

Document Name	**:**	**Sample retrieval**
Document No.	**:**	**ILBS#NLDB:H.19**
Version No.	**:**	**1.0**
Effective Date	**:**	**01/01/2025**

Address

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MATERIALS HANDLING AND DOCUMENTATION

Sample retrieval

Number	Effective date	Pages	Author	Authorized by
ILBS#NLDB:H.19	01/01/2025	7	Mr. Satish Kumar	Dr.Chhagan Bihari
Version	**Review period**	**No. of copies**	**Approved by**	**Date**
1.0	2yrs	3	Dr. Chhagan Bihari	30/12/2024

Location	Subject
Biobank Reception area Almira	Sample retrieval
Function	**Distribution**
To give information about Sample retrieval.	➢ HOD ➢ Biobank Reception area ➢ Master files

SCOPE AND APPLICATION:

This SOP describes the procedures and documentation requirements for sample retrieval, including key considerations and steps to ensure sample quality is maintained.

RESPONSIBILITY:

All qualified NLDB personnel and laboratory staffs are responsible for retrieving samples.

ABBREVIATIONS

Ca	Cancer
OT	Operation Theatre
CT	Chemotherapy
HT	Hormone Therapy
Post NACT	Post-Neoadjuvanvt Chemotherapy
POST RT	Post Radiotherapy
BC	Buffy Coat
PP	Plasma
NT	Normal Tissue
TT	Tumour Tissue
UIN	Unique Identification Number
Rpm	Revolution Per Minute
DNA	Deoxyribo Nucleic Acid
RNA	Ribo Nucleic Acid
MRD	Medical Record Department
EDTA	Ethylenediaminetetra acetic Acid
BRC	Bio samples Release Committee
PAC	Pre Anesthetic Check up
QC	Quality Control
bp	base pairs
DMSO	Dimethyl Sulfoxide
FCS	Fetal Calf Serum
PBMC	Peripheral Blood Mononuclear Cells
RT	Room Temperature (18°-25°C)
EDTA	Ethylene Diamine tetra acetic acid
ACD	Acid citrate dextrose

1.0 PURPOSE

During the operation of a biobank it will be necessary to retrieve samples from freezers for distribution or processing. These procedures deal mainly with retrieval of frozen samples but many points may be applicable to samples stored in other conditions and in other equipment. The purpose of this standard operating procedure (SOP) is to outline procedures that will ensure that retrieval will be conducted under conditions designed to safeguard the quality and integrity of the sample.

2.0 SCOPE

This standard operating procedure (SOP) covers the procedures for sample retrieval and documentation. It outlines general factors that need to be considered during sample retrieval as well as specific steps that need to be followed to maintain the quality of the sample.

3.0 ROLES AND RESPONSIBILITIES

The SOP applies to all qualified NLDB biobank personnel and laboratory staff that are responsible for retrieving samples. This may include the following personnel:

Bio Bank Personnel	Responsibility / Role
Technician/Technologist	Responsible for storing samples, entering data in the informatics system and retrieving samples.

4.0 MATERIALS, EQUIPMENT AND FORMS

The materials, equipment and forms listed in the following list are recommendations only and may be substituted by alternative/equivalent products more suitable for the site-specific task or procedure.

Materials and Equipment	
Inventory Database	
Safety equipment for handling stored samples (such as face mask and thermal gloves for handling liquid nitrogen)	
Pens, markers etc	
Ice	
Dry ice	

5.0 PROCEDURES

Sample retrieval

Sample retrieval requires that the sample be removed from its stable storage environment. Any variation in storage conditions can have a serious effect on the viability of the specimen (if relevant) or cellular and molecular quality of the sample. This retrieval procedure is designed to ensure that the retrieval process maintains the molecular and cellular integrity of the sample. The anticipated use for the samples may determine the best practice to be observed.

5.1 Retrieval – Locating Specimens in Storage

5.1.1 Create a requisition for sample retrieval.

5.1.2 Before transmitting to the biobank check the requisition for accuracy.

5.1.3 Locate specimens to be retrieved on the inventory system.

5.2 Retrieval – Sample Retrieval

5.2.1 At the storage area of the biobank locates and pulls specimens listed on the requisition.

5.2.2 Maintain proper temperature of the specimens according to specimen type; for example, some banks use pre-chilled metal racks on dry ice for sorting frozen vials that have been retrieved. Care must be taken to minimize exposure of the 'source' storage box or tower to ambient temperatures.

5.2.3 Confirm that specimens on the requisition are accounted for in the freezer or storage container.

5.2.4 If missing or incorrect, file a deviation report and attempt to find the samples.

5.2.5 Place retrieved samples in appropriate container or boxes and label appropriately as required for shipping or storage.

5.3 Retrieval – Documentation of Retrieval

5.3.1 Use a checklist to record all steps where appropriate.

5.3.2 Make changes to the inventory system where relevant. If material is released indicate where the sample was shipped. If processed, indicate derivative generated.

5.3.3 If applicable, keep records on the number of times samples may have been thawed and refrozen.

MATERIALS HANDLING AND DOCUMENTATION

Sample retrieval

National
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Standard Operating Procedure

DOCUMENT NAME: MATERIALS HANDLING AND DOCUMENTATION

DOCUMENT NO. :ILBS#NLDB:H

Document Name : Material and Information Handling
Document No. : ILBS#NLDB:H.20
Version No. : 1.0
Effective Date : 01/01/2025

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MATERIALS HANDLING AND DOCUMENTATION

Material and Information Handling

Number	Effective date	Pages	Author	Authorized by
ILBS#NLDB:H.20	01/01/2025	9	Mr. Satish Kumar	Dr.Chhagan Bihari
Version	**Review period**	**No. of copies**	**Approved by**	**Date**
1.0	2yrs	3	Dr. Chhagan Bihari	30/12/2024

Location	Subject
Biobank Reception area Almira	Material and Information Handling
Function	**Distribution**
To give information about the standardized procedures for collection, storage, and management of tissue samples and associated data in the biobank.	➢ HOD ➢ Biobank Reception area ➢ Master files

SCOPE AND APPLICATION:

This SOP applies to the collection, storage, and management of tissue samples and associated data, ensuring proper handling and maintenance of sample quality and value within the biobank.

RESPONSIBILITY:

This SOP applies to ILBS biobank personnel involved in processing, storing, and handling tissue samples, derivative products, and associated data throughout the biobanking process.

ABBREVIATIONS

Ca	Cancer
OT	Operation Theatre
CT	Chemotherapy
HT	Hormone Therapy
Post NACT	Post-Neoadjuvanvt Chemotherapy
POST RT	Post Radiotherapy
BC	Buffy Coat
PP	Plasma
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PAC	Pre Anesthetic Check up
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FCS	Fetal Calf Serum
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RT	Room Temperature (18°-25°C)
EDTA	Ethylene Diamine tetra acetic acid
ACD	Acid citrate dextrose

1.0 INTRODUCTION

Translational research, using advances in molecular biology, archived tissue samples and annotated data, is pursued to aid in the elucidation of the disease process and discovery of new diagnostic and treatment modalities. A collection of stored and well-annotated tissue specimens and derivatives is a valuable resource, important to the research process. The quality of the samples and the extent of the accompanying data is a determinant of value. The goal of the ILBS biobank is to standardize procedures for handling samples and data thus ensuring that the quality and integrity of the collection is consistently maintained at a high level.

2.0 PURPOSE

The ILBS biobank is committed to promoting and educating adherence to high ethical standards and practices in the collection and storage of Human Biological Materials (HBMs) and accompanying information for research purposes. The purpose of policy is to outline general principles that can be used to ensure that HBMs and data are handled and stored in a manner sensitive to the rights of the participant, responsible to the safety of biobank personnel and protective of the quality and integrity of the collection.

3.0 SCOPE

This policy applies to the operational and practical considerations that arise in the process of collecting, storing and maintaining tissue samples and annotated data. The policy is intended to ensure that the goals of the ILBS biobank are met and that the quality and value of the collection is maintained.

4.0 RESPONSIBILITY

This policy applies to ILBS biobank and to biobank personnel involved in all aspects of the tissue biobank program. In particular, it applies to those personnel involved in processing, storing and handling tissue, derivative products and/or accompanying data.

5.0 POLICIES

The use of HBMs and accompanying data is critical for medical research. The public and participants should have confidence that biobanks and researchers will use and handle such material with sensitivity, responsibility and concern for maintaining the value of the collection. The following

principles should guide the ILBS in collecting, processing and storing tissue and information in its custody.

- **Material handling – General Considerations**

The aims to provide users of the tissue biobank standardized, high quality biological samples that are readily accessible for their research needs.

a. To meet the needs of the users, the HBMs will be collected, processed and stored in a manner that optimally maintains the architecture of the tissue and the molecular integrity of the DNA, RNA, and proteins in the specimens.
b. All steps will be performed by staff that are suitably qualified or have adequate training to perform the tasks.
c. Established standard operating procedures (SOPs) will be in place for all procedures involved in collection, processing, storing and retrieving HBMs and annotated information at the biobanks.
d. Laboratory equipment and infrastructure will be appropriate to ensure proper collection, storage, processing, quality control and distribution.
e. Computer/Informatics infrastructure will be appropriate to enable each biobank to collect, store and share data in an efficient and secure method.
f. Quality Assurance (QA) procedures such as routine audits and quality control analysis will be performed to ensure that integrity and quality of the collection is maintained.

- Tissue Collection

a. Tissue for the biobank will be obtained only after all patient diagnostic needs have been met and will be accompanied by documented, informed consent or a waiver of consent from the Ethical committee.
b. Tissue samples will be collected from a wide range of patients (with matched normal specimens whenever possible). The collection process will attempt not to exclude a sub-set of the patient population. If possible, specimens will be collected in sufficient quantity and diversity to be of value in a variety of study designs.
c. Broader molecular profiles can be obtained from samples that have been collected using rigorous and standardized procedures. Collection procedures will be geared to allow use of the samples in genomic and proteomic research.
d. To ensure suitability for genomic and proteomic research the time elapsed between surgical resection and freezing ideally should be rapid to ensure preservation. Adequate documentation should capture the time frame for quality assurance purposes.

- Tissue Processing

a. To ensure suitability for genomic and proteomic research, the processing of the Tissue sample or blood will be done in a manner to protect tissue architecture and the integrity of molecular products.

b. HBMs will be handled as being potentially bio hazardous and laboratory staff should take appropriate precautions when handling tumour tissue or whole blood and blood products.
c. Desiccation and degradation of specimens will be avoided. The method of transport of the tissue sample from the operating room to the pathology or processing lab will be documented.
d. All precautions to avoid cross-contamination of specimens during processing, product isolation or aliquoting will be employed. This should include using fresh containers, pipette tips and blades between specimens and between different areas of the same specimen (e.g. between malignant and associated uninvolved tissue).
e. Snap freezing or freezing in a cryoprotectant will be done by suitable means.
f. Specimens in the collection are useless if incorrectly identified. All samples will be accurately labeled.

➢ Tissue Storage and Retrieval

The storage method of the tissue sample, or derived product, affects the suitability of the sample for use in specific genomic or proteomic studies.

a. Storage procedures will be geared to protecting the integrity of the collection and should allow for efficient and accurate retrieval of samples.
b. Samples will be stored in a manner optimal for their intended category and use. This should be documented.
c. Frozen samples will be stored in screw-capped, plastic containers or cryovials that can be sealed. Vials should permit appropriate labelling, prevention of contamination or samples desiccation and should withstand freezing in liquid nitrogen.
d. Mechanical or liquid nitrogen systems are used for storage of frozen samples, adequate back-up capacity will be in place to ensure that operating temperatures are maintained at all times. Events such as equipment failure or power-outage emergency will be planned for and processes will be in place to deal with possible emergencies.
e. For mechanical freezers, manual defrost feature is optimal as freeze-thaw cycles of automatic units can degrade biologic samples.
f. Ideally, alarm systems should be used to monitor temperatures in the storage freezers and procedures should be in place to permit corrective action before the temperatures falls out of range.
g. Proper procedures will be followed for sample retrieval to ensure that proper conditions are maintained to protect the sample, and that documentation is completed to record any change in inventory.
h. Shipping and transportation procedures will be established to ensure that containers, labels, conditions and methods are optimal for sample protection.
i. Tracking and auditing of HBMs is critical. A high quality inventory will be employed so that every sample can be tracked and audited. All records pertaining to sample retrieval, use, or removal will be maintained to facilitate tracking.

➢ **Informatics – Collection and handling**

- Annotation data (e.g. person, lifestyle, diagnosis, laboratory, clinical and research generated) should be accurate, quality-controlled and standardized as far as possible.
- Data collected may contain common data elements from the following categories including, for example:
  a. Personal
  b. Longitudinal clinical and diagnostic information
  c. Treatment and outcome information
  d. Sample information
  e. Lifestyle and family history
- Computerized inventory and bioinformatics systems used to handle and store annotated data should:
  a. Be responsive to the needs of multiple users
  b. Be available for a long period of time
  c. Use standardized terms to categorize specimens and enter data, across member biobanks
  d. Use an automated data extract system or permit multiple checks of data entry to ensure accuracy
  e. Have the ability to feed back or link standard research results and genomic and proteomic results into the system
  f. Allow for dissemination of information to others as needed
  g. Be searchable at varying levels for certified users
  h. Provide security and access control to ensure privacy rights are protected
  i. Have an inventory management system
  j. Support integration and expansion if needed
  k. Have maintenance features and back-up capabilities

- **Safety considerations**

All personnel coming in contact with HBMs or involved in the operations of the tumour biobank will be trained in safety procedures to minimize injuries to them and protect the material and information held in the biobank. Safety training should be:

a. Given to staff before they begin their work
b. Updated as needed
c. Lead by knowledgeable trainers
d. Appropriate for the background of each employee and to the risks to which each employee is exposed

- Relevant personnel will handle all HBMs as being bio hazardous. The use of liquid nitrogen and dry ice poses specific safety hazards. Appropriate gloves, a face shield and a protective garment will always be used when handling these materials. When dry ice is used, controls to ensure sufficient air and oxygen levels should be ensured.

- Precautions will be taken to minimize risks to injury and damage from biological, chemical, physical, electrical hazards and fire
- Written guidelines will be developed to ensure safety precautions based on national, regional and local regulations.
- Personnel coming in contact with patients and patient information will be trained in maintaining privacy and confidentiality.
- Overall biobank security will be implemented by limiting access of unauthorized personnel to the workplace.

6.0 APPLICABLE REFERENCES, REGULATIONS AND GUIDELINES

- Declaration of Helsinki.http://www.wma.net/en/30publications/10policies/b3/index.html
- Tri-Council Policy Statement 2; Ethical Conduct for Research Involving Humans; Medical Research Council of Canada; Natural Sciences and Engineering Council of Canada; Social Sciences and Humanities Research Council of Canada, December 2010.http://www.pre.ethics.gc.ca/eng/policy-politique/initiatives/tcps2-eptc2/Default/.
- Human Tissue and Biological Samples for use in Research. Operational and Ethical Guidelines. Medical Research Council Ethics Series.http://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC002420
- UKCCSG Guide to Biological Studies Version 1.0, 2002
- US National Biospecimen Network Blueprinthttp://biospecimens.cancer.gov/resources/publications/reports/nbn.asp
- 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
- Qualman, S.J. et al. Establishing a tumour bank: banking, informatics and ethics. Br. J. Cancer (2004). 90-1115-1119.

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Standard Operating Procedure

DOCUMENT NAME: MATERIALS HANDLING AND DOCUMENTATION

DOCUMENT NO. :ILBS#NLDB:H

MATERIALS HANDLING AND DOCUMENTATION

Human Biological sample collection

Document Name	**:**	**Human Biological sample collection**
Document No.	**:**	**ILBS#NLDB:H.21**
Version No.	**:**	**1.0**
Effective Date	**:**	**01/01/2025**

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MATERIALS HANDLING AND DOCUMENTATION

Human Biological sample collection

Number	Effective date	Pages	Author	Authorized by
ILBS#NLDB:H.21	01/01/2025	6	Mr. Satish Kumar	Dr.Chhagan Bihari
Version	**Review period**	**No. of copies**	**Approved by**	**Date**
1.0	2yrs	3	Dr. Chhagan Bihari	30/12/2024

Location	Subject
Biobank Reception area Almira	Human Biological sample collection
Function	**Distribution**
To give information about Human Biological sample collection.	➢ HOD ➢ Biobank Reception area ➢ Master files

SCOPE AND APPLICATION:

This SOP describes the procedure for collection, labelling, and initial handling of human biological samples for biobanking purposes. It applies to all personnel involved in sample collection from consented donors or participants.

RESPONSIBILITY:

This SOP applies to all personnel involved in the collection and handling of human biological samples. Clinical and laboratory staffs are responsible for proper sample collection, labelling, documentation, and ensuring compliance with established procedures.

ABBREVIATIONS

Ca	Cancer
OT	Operation Theatre
CT	Chemotherapy
HT	Hormone Therapy
Post NACT	Post-Neoadjuvanvt Chemotherapy
POST RT	Post Radiotherapy
BC	Buffy Coat
PP	Plasma
NT	Normal Tissue
TT	Tumour Tissue
UIN	Unique Identification Number
Rpm	Revolution Per Minute
DNA	Deoxyribo Nucleic Acid
RNA	Ribo Nucleic Acid
MRD	Medical Record Department
EDTA	Ethylenediaminetetra acetic Acid
BRC	Bio samples Release Committee
PAC	Pre Anesthetic Check up
QC	Quality Control
bp	base pairs
DMSO	Dimethyl Sulfoxide
FCS	Fetal Calf Serum
PBMC	Peripheral Blood Mononuclear Cells
RT	Room Temperature (18°-25°C)
EDTA	Ethylene Diamine tetra acetic acid
ACD	Acid citrate dextrose

MATERIALS HANDLING AND DOCUMENTATION

Human Biological sample collection

1.0 PURPOSE

This SOP describes the clear instructions for Human biological sample collection. A proper management of human biological sample for testing and analysis is essential to assure the quality.

2.0 SCOPE

This SOP guides us for instructions, what procedure should follow and proper handling of Human biological sample without any contamination.

3.0 ROLES AND RESPONSIBILITIES

The SOP applies to all personnel from NLDB that are responsible for assessing the quality of biosamples.

Technician: Processing, storage, records and document outcomes.

OT Incharge: Collect information for future sample collection during surgery

4.0 SAFETY & PRECAUTIONS

- Required Training for processing and Standard laboratory practices
- Risks:Bio fluid exposure
- Required safety equipment
  - Lab coats/scrubs
  - Face shield/safety goggles
  - Closed toed shoes
  - Gloves

- Shoe covers

All staff is expected to be trained and follow universal precautions when handling biological or hazardous materials when performing the any procedures described in the biobank SOPs.

5.0 PROCEDURE

This procedure is intended to ensure that human biological sample will be collected from consented participant in a safe, timely and efficient manner while eliminating the risk of contamination. This is the common procedure for all biosamples.

6.0 PROCESS

Steps	Procedure	Responsibility
1	One day before check the OT list for sample collection.	HOD Biobank
2	Identify the patient	HOD Biobank
3	Contact the patient for informed consent	Technician
4	Coordinate with OT Incharge for Blood and or Tissue collection	OTIncharge/Technician
5	Blood will be collected (8-10 ml)	Technician
6	After the Surgery Tissue sample (without formalin) will be sent to Pathology Department	OTIncharge/Technician
7	Pathology Assistant/technician will gross the Tissue (Immediately)	PathologyAssistant/ Technician
8	Fresh Tissue Pieces will be collected	Technician
9	gross Tissue will be put in formalin	Technician
10	Collected biosamples will be de-identified , processed and stored in biobank	Technician
11	Annotation will be completed and linked with stored samples through LIMS	Technician

National Liver Disease Biobank

Standard Operating Procedure

DOCUMENT NAME: MATERIALS HANDLING AND DOCUMENTATION

DOCUMENT NO. :ILBS#NLDB:H

MATERIALS HANDLING AND DOCUMENTATION

Blood Derivatives

Peripheral Mononuclear Cells from Whole Blood [PBMC] isolation

Document Name : Blood Derivatives: Peripheral Mononuclear Cells from Whole Blood [PBMC] isolation.
Document No. : ILBS#NLDB:H.22
Version No. : 1.0
Effective Date : 01/01/2025

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MATERIALS HANDLING AND DOCUMENTATION

Blood Derivatives

Peripheral Mononuclear Cells from Whole Blood [PBMC] isolation

Number	Effective date	Pages	Author	Authorized by
ILBS#NLDB:H.22	01/01/2025	7	Mr. Satish Kumar	Dr.Chhagan Bihari
Version	**Review period**	**No. of copies**	**Approved by**	**Date**
1.0	2yrs	3	Dr. Chhagan Bihari	30/12/2024

Location	Subject
Biobank Reception area Almira	Blood Derivatives: Peripheral Mononuclear Cells from Whole Blood [PBMC] isolation
Function	**Distribution**
To give information about the standardized procedure for isolation of PBMCs from whole blood samples while ensuring sample quality, viability, and proper handling.	➢ HOD ➢ Biobank Reception area ➢ Master files

SCOPE AND APPLICATION:

This SOP describes the isolation of peripheral blood mononuclear cells (PBMCs) from whole blood using density gradient centrifugation. It emphasizes timely processing of samples to ensure optimal cell viability.

RESPONSIBILITY:

This SOP applies to biobank personnel involved in PBMC isolation from whole blood. Laboratory Technicians are responsible for timely processing, isolation, viability assessment, and proper storage of PBMC samples.

ABBREVIATIONS

Ca	Cancer
OT	Operation Theatre
CT	Chemotherapy
HT	Hormone Therapy
Post NACT	Post-Neoadjuvanvt Chemotherapy
POST RT	Post Radiotherapy
BC	Buffy Coat
PP	Plasma
NT	Normal Tissue
TT	Tumour Tissue
UIN	Unique Identification Number
Rpm	Revolution Per Minute
DNA	Deoxyribo Nucleic Acid
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MRD	Medical Record Department
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bp	base pairs
DMSO	Dimethyl Sulfoxide
FCS	Fetal Calf Serum
PBMC	Peripheral Blood Mononuclear Cells
RT	Room Temperature (18°-25°C)
EDTA	Ethylene Diamine tetra acetic acid
ACD	Acid citrate dextrose

1.0 PURPOSE

This procedure is used for the separation of blood mononuclear cells from human peripheral blood, cord blood or bone marrow collected in anticoagulant using Ficoll-Hypaque density gradient centrifugation.

2.0 SCOPE

Human Mononuclear cells and platelets have a lower density than Ficoll-Hypaque (1.077g/L) and are separated from higher density granulocytes and red blood cells when centrifuged after either under or overlaying the diluted blood on the Ficoll-Hypaque layer. To ensure optimal cell viability and recovery from cryo-preserved material, cells must be isolated as soon as they arrive in the lab. Samples older than 24 hours must have viable cell counts performed to ensure adequate viable cells are stored as cell yield and viability decreases the longer a sample stands.

3.0 ROLES AND RESPONSIBILITIES

This SOP applies to all NLDB staff and collaborative center that are responsible for PBMC isolation.

4.0 MATERIALS, EQUIPMENT AND FORMS

- Phosphate Buffered Saline (PBS) without Ca2+/Mg2+
- Ficoll-Hypaque (Label container with date after opening. Discard Ficoll containers open for more than 6 months).
- Personal safety equipment including gloves, lab coat, and eye protection
- Disposable plastic pipettes in 2ml, 5ml and 10ml graduations
- Hemocytometer and microscope (or automated cell counter).
- Sterile conical centrifuge tubes, 15mL and 50mL
- FCS (or Pooled Human AB Serum)
- Micropipettor with disposable tips
- Trypan Blue Stain
- Laminar Flow Hood (BL2)
- Refrigerated Centrifuge
- Vortex
- Pipette aid.
- Plastic discard bucket.

5.0 SAFETY PROCEDURES & PRECAUTIONS

- Treat all material as infectious,
- All procedures are to be performed in a Biohazard Class II Safety Cabinet
- Lab Coats and gloves must be worn at all times

6.0 PROCEDURES

6.1 All cell preparation will be performed in the Investigator's Lab.

6.2 Add 15 ml Ficoll-Hypaque to a sterile 50 ml conical tube.

6.3 Dilute 15 ml of blood 1:1 with sterile, endotoxin-free PBS (without Calcium and Magnesium).

6.4 Blood should be carefully and slowly overlaid at a ratio of parts diluted blood to parts ficoll reagent in the 50 ml sterile tubes, being careful not to disturb the interface.

6.5 Centrifuge the samples at 900 x g for 20 minutes at room temperature with the break off.

6.6 After centrifugation, remove cloudy interface (PBMC layer) into appropriately labeled 50 ml conical tubes.

6.7 Wash cells by filling tubes with sterile PBS and centrifuge at 250 x g for 10 minutes.

6.8 Decant supernatant after centrifugation, resuspend cells and fill tubes with sterile PBS to count cells.

6.9 Pipette 10 µl of PBMC suspension into a 0.5 ml microcentrifuge tube. Add 90 µl of 0.4% Trypan Blue stain, making a 1:10 dilution (final concentration of Trypan Blue is 0.36%). Mix carefully to avoid aerosol formation.

Dilution Factor: 90 µl Trypan Blue + 10 µl PBMC = 10 /10 µl PBMC

6.10 Load the haemocytometer with cell mixture (Trypan Blue + PBMC's) until the area under the cover slip is sufficiently filled. Make sure to use a cover slip that is specific for the haemocytometer. Allow the cell suspension to settle in the haemocytometer for at least 10 seconds before counting. Count the 4 large corner squares (see diagram below). Viable PBMCs will be colourless; non-viable PBMCs will be blue.

7.0

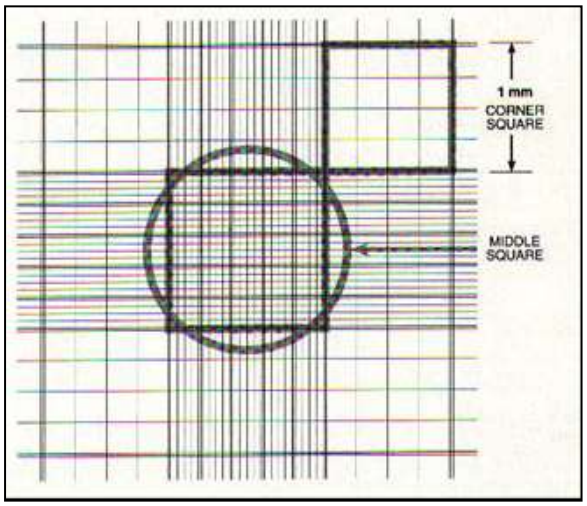

Figure: 3

Count cells in the 4 corner 1 mm squares. Include cells that touch either the top line or left vertical perimeter line of any corner square. Do NOT count any cells that touch either the bottom line or right vertical perimeter line of any corner square.
Calculate the number of PBMC/mL:
10^4 = volume conversion factor to 1 mL
10^1 = specimen dilution factor

$$\text{PBMC/mL} = \frac{\text{PBMC in all four squares} \times 10^4 \times 10^1}{4}$$

7.1 To calculate Cell Viability:

$$\% \text{ Viability} = \frac{\text{Number of Viable Cells Counted} \times 100}{\text{Total Number of Cells Counted}}$$

7.2 To determine the total number of cells, multiply the number obtained above (PBMC/mL) by the cell suspension volume (mL)
Total Cells = PBMC X Volume (mL) of PBMC suspension mL

7.3 Proceed with SOP for freezing PBMC Cells are stored according to the Cell Storage Protocol.

Record:

1. Details of the cell isolation
2. Specimen distribution

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Standard Operating Procedure

DOCUMENT NAME: MATERIALS HANDLING AND DOCUMENTATION

DOCUMENT NO. :ILBS#NLDB:H

MATERIALS HANDLING AND DOCUMENTATION

Blood Derivatives

Peripheral Mononuclear Cells from Whole Blood [PBMC preservation]

Document Name : Blood Derivatives Peripheral Mononuclear Cells from Whole Blood [PBMC preservation]
Document No. : ILBS#NLDB:H.23
Version No. : 1.0
Effective Date : 01/01/2025

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MATERIALS HANDLING AND DOCUMENTATION

Blood Derivatives

Peripheral Mononuclear Cells from Whole Blood [PBMC preservation]

Number	Effective date	Pages	Author	Authorized by
ILBS#NLDB:H.23	01/01/2025	7	Mr. Satish Kumar	Dr.Chhagan Bihari
Version	**Review period**	**No. of copies**	**Approved by**	**Date**
1.0	2yrs	3	Dr. Chhagan Bihari	30/12/2024

Location	Subject
Biobank Reception area Almira	Blood Derivatives Peripheral Mononuclear Cells from Whole Blood [PBMC preservation]
Function	**Distribution**
To give information about the standardized procedure for preservation of PBMC.	➢ HOD ➢ Biobank Reception area ➢ Master files

SCOPE AND APPLICATION:

This SOP applies to all personnel involved in the processing, preservation, storage, and handling of Peripheral Blood Mononuclear Cells (PBMCs) derived from whole blood samples. It is used for maintaining PBMC quality and viability for research, diagnostic, or biobanking purposes.

RESPONSIBILITY:

It is the responsibility of trained laboratory/biobank personnel to perform PBMC preservation, labeling, storage, and documentation as per the established procedure.

ABBREVIATIONS

Ca	Cancer
OT	Operation Theatre
CT	Chemotherapy
HT	Hormone Therapy
Post NACT	Post-Neoadjuvanvt Chemotherapy
POST RT	Post Radiotherapy
BC	Buffy Coat
PP	Plasma
NT	Normal Tissue
TT	Tumour Tissue
UIN	Unique Identification Number
Rpm	Revolution Per Minute
DNA	Deoxyribo Nucleic Acid
RNA	Ribo Nucleic Acid
MRD	Medical Record Department
EDTA	Ethylenediaminetetra acetic Acid
BRC	Bio samples Release Committee
PAC	Pre Anesthetic Check up
QC	Quality Control
bp	base pairs
DMSO	Dimethyl Sulfoxide
FCS	Fetal Calf Serum
PBMC	Peripheral Blood Mononuclear Cells
RT	Room Temperature (18°-25°C)
EDTA	Ethylene Diamine tetra acetic acid
ACD	Acid citrate dextrose

1.0 PURPOSE

This document describes the process for the cryopreservation of peripheral blood mononuclear cell (PBMC) samples.

2.0 SCOPE

These procedures provide practices for the cryopreservation PBMCs for biobanking studies.

3.0 ROLES AND RESPONSIBILITIES

This SOP applies to all NLDB staff and collaborative center that are responsible for PBMC isolation and cryopreservation

4.0 MATERIALS & REAGENTS

- 15ml and 50ml Sterile, Polypropylene, Conical, Centrifuge Tubes
- Sterile pipettes.
- Cryovials (Nalgene/Nunc)

Reagents:

- Human AB Serum (must use tested and pre-approved lot) or FCS
- DMSO
- PBMC suspension

5.0 EQUIPMENT

- Cryofreezing container filled with Isopropanol (Nalgene 5100-0001 or equivalent)
- Centrifuge capable of 200-500 X g speeds.
- -70°C Freezer
- Biosafety Cabinet Hood
- Pipette Aid
- Liquid Nitrogen Freezer
- Dry Ice (option)

6.0 SAFETY PROCEDURES & PRECAUTIONS

- Standard safety operating procedures are to be followed at all times
- Treat all material as infectious,
- All procedures (unless otherwise stated) are to be performed in a Biohazard Class II Safety Cabinet
- Lab Coats and gloves must be worn at all times

7.0 PROCEDURES

7.1 Prepare record in BIMS software and print labels.

7.2 Obtain PBMC suspension from whole blood according to appropriate procedure Ficoll or CPT methods.

7.3 Using a validated automated instrument *(e.g. the Beckman Coulter ViCELL)*, or microscopy counting using Trypan blue to exclude dead cells and contrast to exclude red blood cells. Obtain an accurate count of the total number of viable cells. Record the viability, the cell count and the contaminating RBC.

% Viable Cells: _____

Total # of Viable Cells: _____

7.4 Pellet the cell suspension.

7.5 VERY GENTLY resuspend the cell pellet in room temperature 100% Serum to a final cell concentration of at least 20 x 106/ml *(20 to 50x106/ml)*

7.6 Slowly, drop-by-drop, add to the side of the tube an equal volume of Serum containing 20%DMSO.

7.7 Gently mix cells again avoiding bubbles and IMMEDIATELY aliquot to cryovials*(0.2ml to 1ml/cryovial: minimum volume tested is 0.2ml/cryovial)*.

7.8 The final concentration should be equal or above 106/ml (1 to 3 x 106/30ml were found to be provide the best viability and recovery).

7.9 Aliquot only 0.2-1 ml of PBMC suspension *(~2-10 million cells per vial)* and "*freeze*" *(means put the Nalgene cryodevice @ -80ºC)* IMMEDIATELY

***NOTE: Do not freeze less than 10 million cells per ml.**

- If total cell yield is 30 million cells or more, aliquot 10 million cells per aliquot.
- If total cell yield is less than 30 million cells, aliquot 5 million cells per aliquot.
- Donotdiscardcells: Add any Remaining cell volume to existing cryovial and note the correct number of cells on the label

7.10 Freezing:

7.10.1 Freezing by Cryo-freezing container (Mr. Frosty)

- Add 2-propanol at RT into the cryo-freezing container(Mr. Frosty) Place the cryovials into the container.

- Place the freezing container into a -80°C freezer in a position that will not be moved, jarred, or experience raised temperatures.
- Keep in cryo-freezing Container for a minimum of 12 hours and a maximum of 14 days.
- Transfer cryovials to LN2 freezer on either dry ice or in a pre-chilled LN2 transfer Dewar for storage.
- RECORD LOCATION OF SAMPLES. Alternatively, the cryovials may be shipped on either dry ice or in a pre-chilled liquid N2 Dewar.

7.10.2 Freezing by a Controlled Rate Freezer (CRF) (Planer CRF Series Kryo560)or

the following method may be used to freeze the cells:

- Initiate the Run.
- Close the latch shut the door on the controlled rate freezer.
- Go to the screen panel and press the start button.
- Select the freezing profile from the menu as (per requirement).The demand curve will appear on the screen.
- Enter the No.of samples run in CRF, create profile, and press the Enter button after each entry of the sample Id.
- Once all samples have been entered, press the start button after ensuring Liquid Nitrogen Dewar supply & functions. The freezer will prepare for the run and beep steadily when ready to load.
- When freezer is ready, unlatch and open the door, place the samples inside the CRF.
- Press the start button on the top of the freezer unit. The beeping will stop to indicate the start of the run. The program will continue without further user input for 60-70minutes.
- Record and write the time that the samples were put into the freezer and calculate the elapsed time, in minutes, from the time DMSO & FBS mixture was added to the time they were put in the freezer.
- The elapsed time should be less than 90 minutes.
- Transfer and store cryovials to LN2 freezer
- Record location of samples

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DOCUMENT NAME: MATERIALS HANDLING AND DOCUMENTATION

DOCUMENT NO. :ILBS#NLDB:H

Document Name : Plasma isolation from whole blood.
Document No. : ILBS#NLDB:H.24
Version No. : 1.0
Effective Date : 01/01/2025

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MATERIALS HANDLING AND DOCUMENTATION

Plasma isolation

Number	Effective date	Pages	Author	Authorized by
ILBS#NLDB:H.24	01/01/2025	10	Mr. Satish Kumar	Dr.Chhagan Bihari
Version	**Review period**	**No. of copies**	**Approved by**	**Date**
1.0	2yrs	3	Dr. Chhagan Bihari	30/12/2024

Location	Subject
Biobank Reception area Almira	Plasma isolation.
Function	**Distribution**
To give information about the standardized procedure for isolation of plasma from whole blood samples while ensuring sample quality and proper handling.	➢ HOD ➢ Biobank Reception area ➢ Master files

SCOPE AND APPLICATION:

This SOP applies to all personnel involved in the collection, processing, and isolation of plasma from whole blood samples for biobanking use.

RESPONSIBILITY:

It is the responsibility of trained laboratory personnel to perform plasma isolation, labeling, and documentation as per the defined procedure.

ABBREVIATIONS

Ca	Cancer
OT	Operation Theatre
CT	Chemotherapy
HT	Hormone Therapy
Post NACT	Post-Neoadjuvanvt Chemotherapy
POST RT	Post Radiotherapy
BC	Buffy Coat
PP	Plasma
NT	Normal Tissue
TT	Tumour Tissue
UIN	Unique Identification Number
Rpm	Revolution Per Minute
DNA	Deoxyribo Nucleic Acid
RNA	Ribo Nucleic Acid
MRD	Medical Record Department
EDTA	Ethylenediaminetetra acetic Acid
BRC	Bio samples Release Committee
PAC	Pre Anesthetic Check up
QC	Quality Control
bp	base pairs
DMSO	Dimethyl Sulfoxide
FCS	Fetal Calf Serum
PBMC	Peripheral Blood Mononuclear Cells
RT	Room Temperature (18°-25°C)
EDTA	Ethylene Diamine tetra acetic acid
ACD	Acid citrate dextrose

1.0 PURPOSE

The objective of this procedure is to define the course of action and to establish the basic quality guidelines with respect to collecting, handling and to processing of plasma samples that will be deposited in biobanks belonging to any center or hospital affiliated to the biobank.

2.0 SCOPE

This procedure applies to all plasma samples that are obtained in order to be stored in a biobank.

3.0 ROLES AND RESPONSIBILITIES

The SOP applies to all laboratory personnel from NLDB that are responsible for the processing of blood to obtain blood products for storage in the biobank.

4.0 DEFINITIONS

- **Blood plasma:** liquid and non-cellular fraction of the blood.
- **Pellet**: white little ball of cells (although sometimes it may have a reddish color due to the presence of some erythrocytes). The presence of erythrocytes must be avoided, but it is not sufficient reason to discard the sample.
- **EDTA:**Ethylene diamine tetra acetic acid. Anticoagulant agent that blocks the blood coagulation cascade by attracting ionic calcium.
- **ACD:**Anticoagulant solution of dextrose, sodium citrate and citric acid that inhibits coagulation by attracting ions.
- **Heparin:**Anticoagulant agent that prolongs blood clotting time by activation of antithrombin III.
- **Lipemia:** The presence of lipids in the blood. The plasma takes on a cloudy or milky appearance.
- **Jaundice:**Binding of bilirubin to plasma albumin, leading to intense yellow plasma.
- **Hemolysis**: Destruction of erythrocytes causing the plasma to have a pink or red color.

5.0 MATERIALS, EQUIPMENT AND FORMS

- Sterile Pasteur pipettes
- Cryotubes racks
- 1.5 - 2ml sterile cryotubes
- Markers and ballpoints
- Gloves
- Filter paper
- Lab coat
- Sufficient and appropriate labels for collection tubes

- Label printer
- Cryo storage boxes
- Ultra-freezer -80ºC
-Biobank *Sample management software*

6.0 PROCEDURES

6.1. PRIOR CONSIDERATIONS

Choice of anticoagulant: If the whole blood sample is taken for a specific purpose, it is recommended to choose the type of anticoagulant depending on the type of study/analysis that will be done with the derived samples.

- K2 or K3 EDTA: given their characteristics, they are not recommended for obtaining plasma samples in which tests are to be done that measure the presence of ions or that include divalent cations as reaction intermediates. Conversely, it should be used when the blood sample was obtained for PBMCs or the cell pellet.

- ACD: due to its characteristics it not recommended for obtaining plasma samples in which immunoassays will be done, since it decreases the expected values. Its use is recommended if the blood sample was obtained for obtaining erythrocytes.
- Heparin: because of its characteristics it is not recommended for plasma in which peptide or proteomic analyses are going to be done, because it can interfere with some mass spectrometric analyses. Its use is recommended if the sample was obtained for cellular studies.

6.2 Timing for Blood Collection and Processing

- Preferably, blood collection should be done pre-operation and as close as possible to the time when the tissue is donated to the biobank or at an alternative time, if appropriate for the research study.

- The time requirement for sample processing depends on the intended use and therefore, time to processing should be recorded.

6.3 Time: It is recommended to adjust the optimum processing time of the sample to the type of test that will be done with the sample or its derivatives. According to literatures, the maximum recommended time is:
For studies of biomarkers in plasma, it is advised to centrifuge the blood as quickly as possible (ideally within 30 minutes after extraction) to prevent alterations in the plasma composition due to changes in expression that occur in blood cells as a result of hypoxia.

- For cell assays: 1.5 hours

- For virological studies: 24 hours

- **Temperature:** To determine the temperature that minimizes alteration of the sample, the most important factor to take into account is the estimated time that processing will take (see section 7.1.2).

- **Number of centrifugations:** It is estimated that at least 14% of the peptides that make up the plasma are derived from platelets due to post-extraction activation or to the existence of residual platelets after centrifugation of the blood. The best method to reduce the number of platelets in plasma to <10/nL is to do a double centrifugation. In any case, the requirements of the study for which the sample was taken must be taken into account and the SOP must be adapted accordingly, if necessary, as follows:

- As a general recommendation and for measuring free plasma biomarkers, it is advisable to remove platelets and minimize the release of their contents by double centrifugation. This has been taken into consideration in this SOP.

- In exceptional cases, when measuring biomarkers sequestered by platelets or in specific haematology and cardiology studies related to platelet function, centrifugation conditions must be adapted to the requirements of the study.

- **Storage time:** It is advisable to be aware of the time and temperature at which the samples have been stored, since many components of the plasma are unstable even at -80ºC and become undetectable over time.

- **Data recording:** It is very important to note and record the values of the preanalytical variables considered at this point so that the samples are documented well. Thus, the values of the search parameters can be restricted to the needs of the study for which the sample was requested, which allows us to select more homogeneous samples that give more reproducible results.

7.0. RECEIPT OF THE SAMPLE IN THE BIOBANK

7.1. Check the information and identification of the tubes and ensure the correct relationship between tubes and patient information, in accordance with the confidentiality commitment required by the Data Protection Act. Label and record the sample according to the sample management procedure used by the center.
7.2. It is advised to gather the maximum amount of information possible concerning the sample, both at the time of reception and after processing and storage, and depending on the studies for which they will be used, for example:

- Date and time of receipt and/or processing
- Transport conditions until receipt in the biobank: temperature and time elapsed since its extraction.
- Volume of blood received
- Types of tube and anticoagulant
- Degree of hemolysis
- Degree of Lipemia
- Degree of jaundice
- Degree of Coagulation
- Processing time
- Date and time of freezing

7.3. Register the arrival of the sample and associated data in the computer system.

7.1. COLLECTION OF PLASMA AND STORAGE OF ALIQUOTS

7.1.1 Start from whole blood collected by venipuncture. Processing whole blood to obtain plasma consists of separating the cellular fraction from the blood in order to obtain plasma aliquots that are as representative as possible of the physiological state of the donor at the time it was extracted, and storing them under Biobank conditions to avoid interference in the diagnostic analyses that are to be carried out.

7.1.2 **Temperature:**

- If the blood sample is going to be centrifuged immediately (within 30 minutes after extraction) it is advisable to keep it at room temperature (16-24ºC) until processing, to minimize platelet activation occurring at low temperatures that causes the release of proteins that irreversibly alter the plasma composition.

- If we know that the sample is going to be processed later it is advisable to keep it refrigerated (2-6ºC) until processing, in order to prevent degradation of temperature-sensitive components of the sample.

7.1.3 **Processing time:**
It is recommended that the time between blood collection and freezing is **less than 2 hours**, minimizing as much as possible delays until the first centrifugation. The maximum acceptable time for certain studies is up to 72 hours, if the sample has been stored at 4ºC.
7.1.4 Centrifuge the sample at **1300-1500g for 10 minutes**. To select the temperature, follow the same criteria as outlined in paragraph 7.3.2.
7.1.5 With this first centrifugation plasma is separated from the cellular blood fraction. Three phases will be seen in the tube:

- The upper phase is clear, transparent and yellow, and corresponds to the plasma.
- The intermediate phase, which is very thin and light gray, contains the leukocytes.

7.1.6 Aspirate the plasma (yellow upper phase) carefully with a pipette and transfer it to a properly identified/labelled sterile 15 ml tube.
7.1.7 Centrifuge the transferred material at **2500g for 15 min** at the same temperature as used in paragraph 7.3.4. This second centrifugation eliminates most of the platelets contained in the plasma.

7.1.8 Divide into aliquots of at least 0.5 ml in suitable cryogenic vials that are properly labeled and identified. Close the tubes properly to obtain an airtight seal. Record the number of aliquots obtained for each sample. Store within 2 hours. After obtaining the platelet-poor plasma, it should be kept at 4^0C; otherwise, it must be stored immediately at -80^0C.

7.1.9 Record the location of the sample, as well as the information detailed in section 6.2.3, in the sample management software used by the biobank.

8. REFERENCE DOCUMENTATION

- ***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

- ***Specimen collection and handling: standardization of blood sample collection****. Tammen H. Methods Mol Biol. 2008;428:35-42*

- ***Immunoassay and antibody microarray analysis of the HUPO Plasma Proteome Project reference specimens: systematic variation between sample types and calibration of mass spectrometry data.*** *Haab BB, Geierstanger BH, Michailidis G, Vitzthum F, Forrester S, Okon R, Saviranta P, Brinker A, Sorette M, Perlee L, Suresh S, Drwal G, Adkins JN, Omenn GS. Proteomics. 2005 Aug;5(13):3278-91*

- ***HUPO Plasma Proteome Project specimen collection and handling: towards the standardization of parameters for plasma proteome samples.*** *Rai AJ, Gelfand CA, Haywood BC, Warunek DJ, Yi J, Schuchard MD, Mehigh RJ, Cockrill SL, Scott GB, Tammen H, Schulz-Knappe P, Speicher DW, Vitzthum F, Haab BB, Siest G, Chan DW. Proteomics. 2005 Aug;5(13):3262-77*

- ***Standard Operating Procedures.*** *Australia's Healthy Ageing Biobank. October 2009, version 2.*

-**Standard operating procedure** . Spanish national Biobank network https://brd.nci.nih.gov/brd/sop/download-pdf/1064

- **Standard operating procedure** : Canadian Tissue Repository Network (CTRNet) http://www.ctrnet.ca/resources/operating-procedures

- ***The UK Biobank sample handling and storage protocol for the collection, processing and archiving of human blood and urine.*** *Paul Elliott, Tim C Peakman. International Journal of Epidemiology 2008;37:234–244.*

- ***Stability of plasma analytes after delayed separation of whole blood: implications for epidemiological studies****. Sarah Clark, Linda D Youngman, Alison Palmer, Sarah Parish, Richard Peto, Rory Collins. International Epidemiological Association 2003;32:125-130.*

- ***Serum biobank certification and the establishment of quality controls for biological fluids: examples of serum biomarker stability after temperature variation.*** *ChrastineChaigneau, Thomas Cabioch, Katy Beaumont and FotiniBetsou. Clinical Chemistry and Laboratory Medicine, 2007; 45 (10): 1390- 1395*

9. RELATED DOCUMENTATION

- *SOP for blood extraction*
- *Data collection sheet associated with the sample*
- *SOP quality control*

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Standard Operating Procedure

DOCUMENT NAME: MATERIALS HANDLING AND DOCUMENTATION

DOCUMENT NO. :ILBS#NLDB:H

MATERIALS HANDLING AND DOCUMENTATION

Serum collection and storage

Document Name	**:**	**Serum collection and storage**
Document No.	**:**	**ILBS#NLDB:H.25**
Version No.	**:**	**1.0**
Effective Date	**:**	**01/01/2025**

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MATERIALS HANDLING AND DOCUMENTATION

Serum collection and storage

Number	Effective date	Pages	Author	Authorized by
ILBS#NLDB:H.25	01/01/2025	8	Mr. Satish Kumar	Dr.Chhagan Bihari
Version	**Review period**	**No. of copies**	**Approved by**	**Date**
1.0	2yrs	3	Dr. Chhagan Bihari	30/12/2024

Location	Subject
Biobank Reception area Almira	Serum collection and storage
Function	**Distribution**
To give information about Serum collection and storage.	➢ HOD ➢ Biobank Reception area ➢ Master files

SCOPE AND APPLICATION:

This SOP applies to the collection, processing, and storage of serum samples intended for laboratory or biobanking use.

RESPONSIBILITY:

It is the responsibility of trained laboratory personnel to perform serum collection, processing, labeling, and storage as per the SOP

ABBREVIATIONS

Ca	Cancer
OT	Operation Theatre
CT	Chemotherapy
HT	Hormone Therapy
Post NACT	Post-Neoadjuvanvt Chemotherapy
POST RT	Post Radiotherapy
BC	Buffy Coat
PP	Plasma
NT	Normal Tissue
TT	Tumour Tissue
UIN	Unique Identification Number
Rpm	Revolution Per Minute
DNA	Deoxyribo Nucleic Acid
RNA	Ribo Nucleic Acid
MRD	Medical Record Department
EDTA	Ethylenediaminetetra acetic Acid
BRC	Bio samples Release Committee
PAC	Pre Anesthetic Check up
QC	Quality Control
bp	base pairs
DMSO	Dimethyl Sulfoxide
FCS	Fetal Calf Serum
PBMC	Peripheral Blood Mononuclear Cells
RT	Room Temperature (18°-25°C)
EDTA	Ethylene Diamine tetra acetic acid
ACD	Acid citrate dextrose

MATERIALS HANDLING AND DOCUMENTATION

Serum collection and storage

1.0 PURPOSE

Blood samples are drawn from patients/donors that have been through the informed consent process and agreed to participate in the NLDB biobank program. The objective of this procedure is to define the course of action and to establish the basic quality guidelines with respect to collecting, handling and to processing of serum samples that will be deposited in biobanks belonging to any center or hospital affiliated to the National Biobank.

2.0 SCOPE

This procedure applies to all serum samples that are obtained in order to be stored in a biobank. This protocol does not detail the occupational health and safety processes regarding biohazardous materials and/or chemical products, and it is recommended that the personnel follow the Health and Safety rules established in each center.

3.0 ROLES AND RESPONSIBILITIES

The SOP applies to all laboratory personnel from NLDB biobank that are responsible for the processing of blood to obtain blood products for storage in the NLDB biobank.

4.0 MATERIALS& SUPPLIES

- Syringes and/or material required for collecting blood.
- Evacuated blood collection tubes , SST tubes
- Sterile Pasteur pipettes
- Blood collection tube racks
- Cryotubes racks
- 1.5 - 2ml sterile cryotubes
- Markers and ballpoints
- Gloves to protect staffs who are handling blood and/or biohazardous materials
- Filter paper
- Lab coat to protect against spills and spatters
- Sufficient and appropriate labels for collection tubes

- Label printer
- Cryo storage boxes
- Ultra-freezer -80ºC
- Biobank Sample management software

Timing for Blood Collection and Processing

Preferably, blood collection should be done pre-operation and as close as possible to the time when the tissue is donated to the biobank or at an alternative time, if appropriate for the research study.

The time requirement for sample processing depends on the intended use and therefore, time to processing should be recorded.

5.0 PROCEDURES

5.1 Filled red top blood collection tubes should sit uprights after the blood is drawn at room temperature for a minimum of 30 to a maximum of 60 minutes to allow the clot to form. (Note: the red top tubes do not need to be full.) These tubes, without additives allow the red blood cells to form a clot that includes white blood cells, platelets etc.

5.2 Centrifuge the blood sample at the end of the clotting time (30-60 minutes) in a horizontal rotor for 20 minutes at 1300g at room temperature. If the blood is not centrifuged immediately after the clotting time, the tubes should be refrigerated for no longer than 4 hours.

5.3 Use pipette to transfer the serum into labelled cryovials. Aliquot 100-500µl serum into cryovials. This process should be completed within 1 hour of centrifugation. (Note: Be very careful not to pick up red blood cells when aliquoting. This can be done by keeping the pipet above the red blood cell layer and leaving a small amount of serum in the tube).

5.4 Place all aliquots upright in a specimen box or rack in -80°C or colder freezer. All specimens should remain at -80°C or colder.

- **Storage time:** It is advisable to be aware of the time and temperature at which the samples have been stored.

➢ **Data recording:** It is very important to note and record the values of the preanalytical variables considered at this point so that the samples are documented well. Thus, the values of the search parameters can be restricted to the needs of the study for which the sample was requested, which allows us to select more homogeneous samples that give more reproducible results.

6.0 RECEIPT OF THE SAMPLE IN THE BIOBANK

6.1 Check the information and identification of the tubes and ensure the correct relationship between tubes and patient information, in accordance with the confidentiality commitment required by the Data Protection Act. Label and record the sample according to the sample management procedure used by the center.
6.2 It is advised to gather the maximum amount of information possible concerning the sample, both at the time of reception and after processing and storage, and depending on the studies for which they will be used, for example:

- Date and time of receipt and/or processing
- Transport conditions until receipt in the biobank: temperature and time elapsed since its extraction.
- Volume of blood received
- Types of tube and anticoagulant
- Degree of Coagulation
- Processing time
- Date and time of freezing

6.3 Register the arrival of the sample and associated data in the computer system.

7. REFERENCE DOCUMENTATION

-***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

- ***Specimen collection and handling: standardization of blood sample collection***. *Tammen H. Methods Mol Biol. 2008;428:35-42*

- ***Standard Operating Procedures.*** *Australia's Healthy Ageing Biobank. October 2009, version 2.*

-**Standard operating procedure**. Spanish national Biobank network
https://brd.nci.nih.gov/brd/sop/download-pdf/1064
-**Standard operating procedure** : Canadian Tissue Repository Network (CTRNet)
http://www.ctrnet.ca/resources/operating-procedures
- ***The UK Biobank sample handling and storage protocol for the collection, processing and archiving of human blood and urine.*** *Paul Elliott, Tim C Peakman. International Journal of Epidemiology 2008;37:234–244.*

- ***Serum biobank certification and the establishment of quality controls for biological fluids: examples of serum biomarker stability after temperature variation.*** *ChrastineChaigneau, Thomas Cabioch, Katy Beaumont and FotiniBetsou. Clinical Chemistry and Laboratory Medicine, 2007; 45 (10): 1390- 1395*

8. RELATED DOCUMENTATION

- *SOP for blood extraction*
- *Data collection sheet associated with the sample*
- *SOP quality control*

National
Liver
Disease
Biobank

Standard Operating Procedure

DOCUMENT NAME: MATERIALS HANDLING AND DOCUMENTATION

DOCUMENT NO. :ILBS#NLDB:H

Document Name : Error Management Program
Document No. : ILBS#NLDB:H.26
Version No. : 1.0
Effective Date : 01/01/2025

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ILBS#NLDB:H.26	01/01/2025	9	Mr. Satish Kumar	Dr.Chhagan Bihari
Version	**Review period**	**No. of copies**	**Approved by**	**Date**
1.0	2yrs	3	Dr. Chhagan Bihari	30/12/2024

Location	Subject
Biobank Reception area Almira	Material and Information Handling
Function	**Distribution**
To give information that the employee who discovers a variance or deviation is responsible for completing the Variance Report (hereafter referred to as "report") and notifying his or her supervisor about the incident.	➢ HOD ➢ Biobank Reception area ➢ Master files

SCOPE AND APPLICATION:

This SOP covers a standard approach to managing non-conformances and accidents at Institute of Liver & Biliary Sciences Biobank. It provides corrective and preventive measures to prevent deviations from recurring in the system.

ABBREVIATIONS

Ca	Cancer
OT	Operation Theatre
CT	Chemotherapy
HT	Hormone Therapy
Post NACT	Post-Neoadjuvanvt Chemotherapy
POST RT	Post Radiotherapy
BC	Buffy Coat
PP	Plasma
NT	Normal Tissue
TT	Tumour Tissue
UIN	Unique Identification Number
Rpm	Revolution Per Minute
DNA	Deoxyribo Nucleic Acid
RNA	Ribo Nucleic Acid
MRD	Medical Record Department
EDTA	Ethylenediaminetetra acetic Acid
BRC	Bio samples Release Committee
PAC	Pre Anesthetic Check up
QC	Quality Control
bp	base pairs
DMSO	Dimethyl Sulfoxide
FCS	Fetal Calf Serum
PBMC	Peripheral Blood Mononuclear Cells
RT	Room Temperature (18°-25°C)
EDTA	Ethylene Diamine tetra acetic acid
ACD	Acid citrate dextrose

1.0 PURPOSE:

1.1 The purpose of this procedure is to define the method for investigating, documenting, and reporting any in-date product or process that may adversely impact the biosample quality. This procedure describes how non-conformances and accidents that occur within the control of Institute of Liver & Biliary Sciences (ILBS)are identified, investigated, and resolved. Immediate corrective and preventive actions are implemented to improve processes.

1.2 A root cause analysis or thorough investigation is conducted for non-conformances. Accepted industry methods for root cause analysis may be used to identify the underlying causes of non-conformance.

1.3 Non-conformances and accidents are monitored and analyzed to evaluate process improvement, and documentation is maintained as per the procedure mentioned in this SOP.

2.0 SCOPE:

2.1 This SOP covers a standard approach to managing non-conformances and accidents at Institute of Liver & Biliary Sciences Biobank. It provides corrective and preventive measures to prevent deviations from recurring in the system.

3.0 DEFINITIONS:

3.1 Variance – Any variationin process, supplies, product or service with respect to one or more specified requirements outlined inInstitute of Liver & Biliary SciencesSOPs or processes. It includes non-conformances, deviations, OOS,accidents, and adverse reactions.

3.2 Non-conformance – A product or service that does not satisfy one or more specified requirements such as unacceptable samples, test run, procedure, process, reagent, material, or equipment that is not functioning within pre-established methods, parameters, or specifications(i.e. failure to meet requirements), with the potential to impact product quality, safety, efficacy, or data integrity.

3.3 Deviation – An unusual or abnormal event, formally defined as a departurefrom approvedpolicies, processes or procedures. It can be planned or unplanned. It includes any variation, discrepancy or out of specification related to processes, supplies, products, results or services with respect to one or more specified requirements outlined in ILBS SOPs or applicable standards. Not all deviations result in an unacceptable product or result.

3.4 OOS – Out of Specification result is a specific type of deviation pertaining to Quality Control (QC) testing, where the results fall outside pre-determined acceptance criteria. OOS does not apply to routine in-process testing, where tests are performed to monitor and/or adjust the process until the end-point is achieved. However, it applies to stability testing and requires the investigationof significant atypical trends.

3.5 Accident – An unplanned incident that may or may not affect product quality.

3.6 Root Cause Analysis – Asystemic process of describing the problem to identify all the possible causes. It includes developing and implementing a solution and an action plan.

3.7 Preventive Action (PA) – A future-oriented plan or activity implemented to eliminate the potential cause of non-conformances or other undesirable situations.

3.8 Corrective Action (CA) – A measure taken to eliminate the cause of an existing non-conformance, deviation, accident, or other undesirable situation(s).

4.0 RESPONSIBILITIES:

4.1 The employee who discovers a variance or deviation is responsible for completing the Variance Report (hereafter referred to as "report") and notifying his or her supervisor about the incident.

4.2 The discoverer of the incident, with the assistance of the immediate supervisor, is responsible for performing immediate corrective action.

4.3 The process owner of the procedures affected by the incident is responsible for investigating the non-conformance, performing a root cause analysis, and notifying Facility In-charge if biosamples are involved.

4.4 Execution of corrective action and preventive action is also the responsibility of the process owner.

4.5 Quality Assurance is responsible for the following follow-up actions with regards to the report:

4.6 Assign a Quality Assurance (QA) report number to the report.

4.7 Review the report for an accurate description of the incident and attachment of supporting documents.

4.8 Planning and follow-up on the effectiveness of the corrective action/preventive action plan.

4.9 If biosample quality is affected, review to ensurefinal product disposition is approved by the Facility In-charge.

4.10 Quality Assurance is responsible for tracking variances using, "Variance Tracking Log."

4.11 Quality Assurance is responsible for submitting periodic statistical reports on variances to Executive Management.

5.0 REFERENCES:

5.1 ISO Standards 9001:2008

5.2 NABL Standards ISO 15189:2007

6.0 SAFETY:N/A

7.0 MATERIALS: N/A

8.0 PROCEDURE:

8.1 When a non-conformance, a variance or deviation from practice, or an accident occurs, the discoverer (Technical staff) in any department of ILBS Burbank begins by opening a "Variance and Out of Specification Report" (**Form ILBS#NLDB:H.26-1**).

8.1.1 Complete CAPA Form by filling in the Variance Type. Check appropriate box- Unplanned, Planned, Accident or Adverse Event.

8.1.2 Complete CAPA Form with Identification of the report by filling in the date of variance, date of discovery, and reporting date, reporting employee(name). Enter product/equipment/ reagent/ supply involved, write Equipment ID#, or Lot# or Product ID# and expiration date (if applicable).

8.1.3 Complete CAPA Form with description of what occurred, date and time, area of occurrence, how was the variance discovered, acceptable criteria if applicable, contributing factors (if known), responsible staff and any additional information, SOPs involved or applicable and persons contacted with date and time (if applicable).

8.1.4 The reporting employee or any other staff may take immediate corrective action. Complete CAPA Form, enter immediate action taken, check appropriate box, date and time(if applicable).

8.1.5 IMPORTANT: If the incident compromised a biosample, notify the Facility In-charge immediately.

8.1.6 If the acceptance criteria of biosamples are involved, facility In-charge determine final disposition of the biosamples in CAPA Form.

8.1.7 The supervisor investigates the variance and completes CAPA Form, noting the details of investigation andIf no investigation performed, justify why? The investigating supervisor initials/Signature and dates to be filled at the end of the section.

8.1.8 QA determines the final category of the variance, the root cause and completes CAPA Form (refer SOP H)

8.1.9 QA with (if needed) or without consulting operations management, completes CAPA Form, describes the Corrective Action(s) and Preventive action taken or planned to prevent recurrence of the problem or potential for non-conformance, including time frames. If no CAPA is taken or planned justify why?

8.1.9.1 Determine expected completion date, responsible staff to implement CAPA.

8.1.10 QA verifies implementation and effectiveness of CAPA. If yes, close variance report.

8.1.10.1 QA initial or signature and dates under CAPA Form.

8.1.10.2 If CAPA is not effective explain. QA initiates follow-up plan for corrective action in consultation with Facility In-charge.

8.1.11 The Facility In-charge completes CAPA Form,

8.1.11.1 If biosamples are involved and final disposition is to be determined by Facility In-charge and determines if biosample is non-conforming.

8.1.12 When complete, QA signs off in CAPA Form and files the original in the QA location of “Variance Report Binder”.

8.1.13 The closure date may be written in immediately, if known, or left blank until an appropriate closure date is determined.

8.1.14 Review the preventive action plan after an appropriate period of time to evaluate if the plan was effective. (Evaluation can be in the form of an assessment).Document the evaluation's findings in CAPA Form of the report and record the closure date.

8.1.15 When complete, file the original in the QA file for non-conformance reports.

9.0 EXPECTED RESULTS:

9.1 Non-conformances and accidents are investigated, reviewed, and remedied using immediate corrective and preventive action. Appropriate follow-up and documentation are performed.

10.0 ATTACHMENTS:

10.1 Attachment A: Variance Category and Root Cause

11.0 RECORDS / OBJECTIVE EVIDENCE:

11.1 All completed records (forms) will be stored in a manner that will facilitate effective retrieval and prevent damage or deterioration.

FORM TITLE	FORM NUMBER	LOCATION	CUSTODIAN	RETENTION
Variance/Out of Specification Report	**ILBS#NLDB:H.26-1**	Variance Report Binder	Quality Assurance	Indefinitely

12.0 DOCUMENT REVISION HISTORY:

Revision Number	Description of Changes
.1	N/A; New Document

Attachment A: Variance Type &Category and Root Cause

VARIANCE TYPE
Deviation
Out of Specification
Accident
Adverse Event
Other Undesirable
Planned / Unplanned

VARIANCE CATEGORY	
DO	Documentation
EQ	Equipment
MR	Material/Reagent
PN	Personnel
PR	Product
LE	Labeling Error
PRO	Procedure
USC	Unacceptable Shipping Conditions
OTH	Other/Not Applicable

ROOT CAUSE	
ILBSOE	ILBS Employee Operator Error
NOE	Non-Employee Error
TR	Training Insufficient
SOP1	SOP Not Followed
SOP2	SOP Inadequate
RI	Record Incorrect/ Incomplete
MAL	Equipment Malfunction
CSE	Computer Software/ Data Entry Error
WEA	Weather
OTH	Other/Not Applicable