Title	Biopsy Collection and Processing for Clinical Trials
SOP Code	SOP205_02
Effective Date	04-Jan-2016

Approvals

Name and Title (typed or printed)	Signature	Date dd/Mon/yyyy

1.0 PURPOSE

This Standard Operating Procedure (SOP) describes the process for collecting, processing, and storing tumour biopsies for clinical research.

2.0 SCOPE

Paired tumour biopsies (before and after treatment) may be used to assess the molecular status of the participant's tumour to obtain a better understanding of biomarkers involved in the proposed drug pathway. Tissue specimens are collected from participants that have been through the informed consent process. This procedure is intended to ensure that fresh tumour biopsy specimens will be collected from participants in a safe, timely, and efficient manner then suitably processed to ensure high integrity and high quality specimens.

3.0 RESPONSIBILITIES

This SOP applies to clinical research personnel involved in collection and processing of research biopsies. Roles and responsibilities may vary at specific sites.

4.0 DEFINITIONS

See Glossary of Terms.

5.0 PROCEDURE

5.1 General Considerations

5.1.1 The scientific utility of the data obtained from the analysis of tissues is directly related to the quality of the tissue specimen.

5.1.2 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.

5.1.3 Never place tissue intended as a fresh frozen specimen in formalin.

5.1.4 Use cryovials suitable for submersion in liquid nitrogen.

5.1.5 Note: Collection and processing procedures may differ, if specified in the instructions provided in the study-specific laboratory manual from the sponsor

5.2 Biopsy Collection

5.2.1 Ensure that biopsies are only performed by qualified personnel.

5.2.2 Biopsy Team: Notify the person responsible for specimen handling/transport at least 30 minutes prior to the procedure, to allow time for bringing necessary pre-labeled collection and transportation supplies to the biopsy room.

5.2.3 Designate a person who will be responsible for obtaining the specimen from the biopsy team.

5.2.4 Specimen Handler: Present proper identification to the biopsy room staff to acquire the specimen.

5.2.5 Ensure that specimen transportation to the pathology laboratory or designated processing laboratory is arranged, in a manner optimal for preservation of cellular and molecular integrity.

5.2.6 Ensure that all materials and equipment for collection and processing are ready for use. Prelabel the necessary cryovials, cryomolds, RNAlater® later tubes, or media containers.

5.2.7 Obtain core biopsies using a minimum of an 18G needle. Collect as many cores as possible to ensure an adequate yield of viable tumour cells.

5.2.8 Record the time of tissue collection. No more than 30 minutes should elapse between the time of biopsy, processing of a specimen.

5.2.9 Fix specimens in formalin or snap freeze, as soon as possible after harvesting, unless intended for another method of preservation.

5.2.10 Ensure that the tissue never desiccates or is contaminated by surrounding tissue or other samples. If required, saline can be used to keep tissue moist.

5.3 Processing Specimens Fixed in Formalin for Paraffin Embedding

5.3.1 Immediately transfer the biopsy material into a specimen jar, containing approximately 30ml of 10% neutral buffered formalin. Small or soft biopsies may be contained in a biopsy bag or tissue cassette for maximum retrieval of specimen.

5.3.2 Perform fixation at room temperature (25º C). Record the time and duration of fixation; optimally, duration of fixation should be 16-24 hours, but no more than 48 hours.

5.3.3 Transfer the specimen to the laboratory for paraffin-embedding and tissue sectioning, performed using standard histopathologic laboratory techniques.

5.3.4 Optional: Transfer biopsies to 70% ethanol after formalin fixation, and ship to central laboratories for paraffin embedding.

5.4 Processing Specimens Snap-Frozen in Liquid Nitrogen, Suitable for Extraction of DNA, RNA, and Protein

5.4.1 Note: Do not place the specimen in contact with formalin or serum at any point in the process.

5.4.2 Place the biopsy material into a cryovial, using sterile forceps.

5.4.3 Close the cryovial.

5.4.4 Submerge the cryovial in liquid nitrogen for approximately 30-60 seconds or as per institutional guidelines. Do not freeze the tissue directly on ice.

5.4.5 Record the time of freezing.

5.4.6 Place samples on dry ice to be carried to the -80˚C freezer, or liquid nitrogen storage facility.

5.4.7 Storage in liquid nitrogen: Place the samples in the vapour phase of liquid nitrogen.

5.4.8 Record the storage location and time of storage.

5.5 Specimens Frozen in Optimal Cutting Temperature (OCT) Compound, Suitable for Producing Frozen Tissue Sections

5.5.1 Place a few drops of the OCT compound into a pre-labelled plastic cryomold.

5.5.2 With sterile forceps, place the biopsy onto the OCT in the cryomold. If relevant to your type of tumour specimen, orient the tissue in the cryomold.

5.5.3 Add more OCT to cover the tissue and fill the cryomold.

5.5.4 Avoid introducing any air bubbles into the OCT. Use forceps or a transfer pipette to release any bubbles that may become trapped around the tissue.

5.5.5 Using forceps, submerge the cryomold in liquid nitrogen until the OCT media turns white; approximately 60 seconds.

5.5.6 Remove the cryomold from the liquid nitrogen.

5.5.7 Record the time of freezing.

5.5.8 Wrap the cryomold in aluminum foil, or place the cryomold into a small labelled zip-lock bag.

5.5.9 Place samples dry ice to be carried to the -80˚C freezer, or liquid nitrogen storage facility.

5.5.10 Storage in liquid nitrogen: Place the samples in the vapour phase of liquid nitrogen.

5.5.11 Record the storage location and time of storage.

5.6 Specimens Frozen in RNA Later, Suitable for RNA Extraction.

5.6.1 Immediately transfer the biopsy material into a cryovial containing RNA later, at room temperature.

5.6.2 Record the time the biopsy was placed in RNA later.

5.6.3 Store RNA later specimens at 4˚C overnight, before storage in a -80˚C freezer or liquid nitrogen storage facility.

5.6.4 Storage in liquid nitrogen: Place the samples in the vapour phase of liquid nitrogen.

5.6.5 Record the storage location and time of storage.

5.7 Fresh Specimens, Suitable for Cell Culture, Flow Cytometry, Xenograft, etc.

5.7.1 Note: Collection and processing procedures may differ, if specified in the instructions provided in the study-specific laboratory manual from the sponsor.

5.7.2 Immediately transfer the biopsy material into a specimen jar containing approximately 30ml of suitable media, such as PBS or RPMI.

5.7.3 Record the time the biopsy was placed in media.

5.7.4 Processing personnel: Transfer the biopsy specimen to the laboratory for further processing and analysis.

5.8 Storage

5.8.1 Store tumour specimens according to protocol or other study document until shipment is required.

5.8.2 Store all tumour biopsy specimens in boxes clearly labelled with the protocol number, in numerical order of participant study number.

5.8.3 Record the collection, processing, and location information in the specimen inventory.

6.0 REFERENCES

Health Canada, Food and Drug Regulations, Part C, Division 5, Drugs for Clinical Trials Involving Human Subjects, (Schedule 1024), June 20, 2001.

Health Canada, Guidance for Industry, Good Clinical Practice: Consolidated Guideline, ICH Topic E6, 1997.

2011 NCI Best Practices for Specimen Resources. Office of Biorepositories and Biospecimen Research, National Cancer Institute, Bethesda, MD. http://biospecimens.cancer.gov/bestpractices/2011-NCIBestPractices.pdf

ISBER Best Practices for repositories: Collection, storage, retrieval and distribution of biological materials for research, 3rd Edition, 2012 http://www.isber.org

CTRNET Standard Operating Procedures, Canadian Tissue Repository Network

7.0 REVISION HISTORY

SOP Code	Effective Date	Summary of Changes
SOP205_01	01-Aug-2012	Original version
SOP205_02	02-Jan-2016	5.1.5, 5.4.4: Added note for use of procedures according to study specific manual. 5.7.1: Removed note for use of procedures according to study specific manual. 5.2.6: Added Registered designation for commercial tubes. 5.4.2: Removed instruction for numerous vials. 5.5.2: Changed from clean to sterile forceps. Updated references. Removed OTRN logo.