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| AIDS and Cancer Specimen Resource (ACSR) | Effective Date: August 2018 |
| Technical: Solid Tissue Collection | Version 2.0 |
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1.0 PURPOSE

The purpose of this document is to establish the procedure to bank solid tissues for the AIDS and Cancer Specimen Resource (ACSR).

2.0 SCOPE

This standard operating procedure (SOP) describes methods for tissue processing and storage. This SOP applies to all personnel from ACSR Regional Biospecimen Repositories (RBRs) and affiliates that are responsible for performing tissue procurement for the ACSR. The ACSR RBRs and affiliates process and bank biospecimens under site specific approved Human Subjects Protocols. Biospecimens, Samples, Aliquots and Derivatives are entered into the ACSR database when consent for banking and research use have been verified by the Protocol PI or ACSR designee. Each RBR and affiliate site is responsible for Human Subjects compliance as per their institutional guidelines and their local approved Human Subjects Protocol. This SOP does not cover detailed safety procedures for handling biohazardous material and it is recommended that personnel follow institutional biosafety guidelines.

3.0 REFERENCE TO OTHER ACSR SOPS OR POLICIES

ACSR SOP Tech009 Biospecimen Handling

ACSR SOP Tech010 Biospecimen Labeling

4.0 DEFINITIONS

| Term/Acronym | Definition |
|-----------------|---|
| ACSR | AIDS and Cancer Specimen Resource |
| ACSR Management | ACSR staff designated by their official job title and descriptions as a Principal Investigator, Manager or Director. |
| Aliquot | The sample has the original characteristics of the original or parent specimen but in smaller quantities (1 FFPE block vs unstained sections from the FFPE block) |
| Biospecimen | Human material such as urine, blood, tissue stored in a biorepository for use in laboratory research. For the ACSR this is considered the original or parent biospecimen. |
| Derivative | The original characteristics of the specimen are changed (FFPE vs DNA derived from FFPE). |

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| FFPE | Formalin Fixed Paraffin Embedded |
| OCT | Optimal Cutting Temperature Compound |
| PBS | Phosphate Buffered Saline |
| PPE | Personal Protective Equipment such as gloves, lab coat, face shield. |
| RBR | Regional Biorepository |
| RPMI | Roswell Park Memorial Institute Media |
| Sample | This is an aliquot, derivative or if the Parent Specimen received is stored as a whole specimen, it is referred to as a sample, as per ACSR database definition. |
| SOP | Standard Operating Procedure. A set of written instructions that document a routine or repetitive activity followed by an organization. |
| Specimen | The same as Biospecimen. Human material such as urine, blood, tissue stored in a biorepository for use in laboratory research. For the ACSR this is considered the original or parent biospecimen/ specimen. |
| Tissue Transport Vessels | A sterile container used to transport biospecimens such as: urine cup, draw tube, conical tube or capped vessel. |
| Universal Precautions | This is an approach to infection control to treat all human blood and certain human body fluids as if they were known to be infectious for HIV, HBV and other bloodborne pathogens |

5.0 ROLES AND RESPONSIBILITIES

This SOP applies to all personnel from ACSR RBRs and affiliate sites that are responsible for biobanking tissue.

| ACSR RBR Personnel | Responsibility/Role |
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| ACSR Staff Member | Transport tissue, process biospecimen, label vials, data entry and record storage. |

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6.0 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 | Materials and Equipment (Site Specific or equivalent) |
|---|---|
| Biospecimen form | |
| Personal Protective Equipment (PPE) | Gloves, gown/scrubs, lab coat, face shield, etc. as appropriate for the environment. |
| Cryo marking pen, pre-labeled container, or preprinted labels. | Biospecimen labels may be hand written on the biospecimen container (Statmark #SMP-BK). Pre-printed labels or pre-labeled containers may be used. |
| Sterile biospecimen container | VWR# 15704-085 or Fisher #14-828-320 |
| Clean forceps | |
| Clean scalpel blades | Fisher #31-200-32 or VWR # 21909-654 |
| Aluminum foil | |
| Laboratory gloves | VWR #82026-426 or Fisher #19-130-1597C |
| Needle/sharps disposal unit | Fisher #14-827-63 or VWR#19001-005 |
| Sterile saline | 0.9% sodium chloride |
| Phosphate Buffered Saline (PBS) | cellgro #21-040-CV |
| Dewar | A vacuum device used for transporting or using liquid nitrogen |
| RPMI cell culture media | cellgro 15-040-CV |
| Various cooling materials in appropriate containers. (wet ice, dry ice, liquid nitrogen and/or isopentane.) | Freezing processes vary at different sites. Each site follows these procedures to maintain high quality molecular integrity. After resection, prior to freezing keep tissues moist and cool. Tissues should be frozen within 1 hour of resection. Tissues should be snap-frozen in liquid nitrogen with or without isopentane. Tissues should never be frozen on dry ice. |
| Optimal Cutting Temperature Compound | OCT Tissue Tek #4583 |

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| Appropriate storage containers | The storage container is site specific. Biospecimens may be snap frozen in liquid nitrogen and stored in a cryovial, or they might be frozen in OCT and wrapped in foil and stored in a cryo-cassette (Provia PSC-1-Proviasette or cardboard pill boxes such as Argos Technologies #89085-506). |
| -80 freezer | |

7.0 PROCEDURES

This procedure is intended to ensure that tissue is processed in a safe and efficient manner while eliminating the risks of contamination and loss.

7.1 SPECIAL SAFETY PRECAUTIONS

- 7.1.1 Comply with "Universal Precautions" when handling all biospecimens.
- 7.1.2 Use PPE in accordance with the institution's guidelines.
- 7.1.3 Standard best-practice working procedures include careful manipulation of the patient biospecimens, disinfection of countertops and equipment used during testing, and disposal of biohazard waste into appropriate receptacles.

7.2 Verification of Identification Information on Tissue Transport Vessels

As applicable, verify the accuracy of coded patient information (in keeping with privacy and ethical policies) and ensure that it corresponds with the label information on transport vessels. Ensure that all personnel are trained in the use of the ACSR database and local electronic information system(s).

7.3 General Considerations

- 7.3.1 Prepare Biospecimen form and tissue transport vessels.
- 7.3.2 Biospecimen form information may include: coded patient identifiers, date and time of collection, warm ischemia time, cassette/vial identifiers, and the final location of the cassette or vial in the inventory.
- 7.3.3 Treat all tissue as potentially infectious and use appropriate PPE.

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- 7.3.4 The tissue is placed in a sterile container and preferably on gauze saturated with saline or phosphate buffered saline solution. Drying can create artifact in the biospecimen.
- 7.3.5 The container should have coded identifier, date, time and accompanying paperwork identifying tissue and disease/status.
- 7.3.6 The time between resection and freezing should be less than 1 hour.
- 7.3.7 If there will be a delay before the tissue is prepared by the ACSR staff member, place the biospecimen in a refrigerator or place the biospecimen container on ice. Cold slows down autolysis and preserves tissue. Be sure to saturate the tissue with saline, PBS, RPMI or other appropriate media.
- 7.4 Optimal Cutting Temperature (OCT) Compound for freezing of tissue
 - 7.4.1 Fill a wide mouth dewar 1/3 full with liquid nitrogen.
 - 7.4.2 Fill a 250 ml metal beaker halfway with isopentane.
 - 7.4.3 Using long 12" forceps hold the beaker and very slowly lower it into the wide mouth dewar until it touches the bottom.
 - 7.4.4 Stir the isopentane using the -150⁰C thermometer and keep stirring until the isopentane is quenched to -150⁰C.
 - 7.4.5 Remove the thermometer.
 - 7.4.6 Trim tissue to a size no larger than 12mm² and 4mm thick, or if the tissue is smaller no trimming is necessary and can be frozen "as is".
 - 7.4.7 Fill an embedding cryo-mold half way with OCT compound.
 - 7.4.8 Place tissue on top of OCT in tissue cryo-mold, cover the tissue with a layer of OCT until the mold is filled.
 - 7.4.9 Label the lip of the cryo-mold with block number, initials, or tissue type if more than one patient or more than one organ is being process, bend up the lip of the mold.
 - 7.4.10 Repeat until all tissue is placed in cryo-molds and covered with OCT.
 - 7.4.11 Do not let tissue sink to the bottom of the cryo-mold.

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Correct



Incorrect

- 7.4.12** Using the long 12" forceps grab the handle of the cryo-mold that contains the biospecimen and submerge it into the isopentane bath. **DO NOT RELEASE MOLD.**
- 7.4.13** Freeze in isopentane for 10 seconds. Remove mold with tissue now frozen solid. Place the tissues in cryostat at -20°C or in a container with dry ice until ready to store all biospecimens.
- 7.4.14** Monitor the temperature of the isopentane. Warm or cool as necessary to maintain -150°C .
- 7.4.15** Pop block out of cryo-mold, wrap in chilled aluminum foil that has been labeled with coded identifiers.
- 7.4.16** Put foil wrapped blocks in labeled and chilled cryo-cassette, box or cryo-bag. Store in -70 to -80°C freezer. Biospecimens will keep for years if stored properly.
- 7.5** Snap freeze tissue in liquid nitrogen (LN_2)
 - 7.5.1** Different procedures result in varied amount of tissue available for biobanking. If the available tissue (tumor and matched normal, if possible) is less than 100mg ($\sim 5\text{mm}^3$) then freeze tissue in a single cryovial. If the tissue is greater than 100mg, then cut the tissue in aliquots less than 100mg and collect in multiple vials. The tissue should be cut to allow the tissue to be frozen free inside the cryovials. This allows for the tissue to be easily accessed (it drops out of the vial) for a portion to be cut off and returned to the vial for storage.
 - 7.5.2** Annotate biospecimen form and label vials with coded identifiers.
 - 7.5.3** Drop vial in LN_2 for 2 minutes.
 - 7.5.4** Transfer the vial to -80°C freezer for long term storage.
- 7.6** Formalin Fixed Paraffin Embedded (FFPE) processing of tissue

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- 7.6.1 Tissue might also be collected and fixed. This tissue is used to assess the quality of the frozen biospecimen.
- 7.6.2 For any tumor biospecimen, a portion (10-20%) is placed in a tissue cassette.
- 7.6.3 If multiple vials are collected, a representative portion should be collected for each vial or a representative portion should be collected for paraffin embedding as quality control.
- 7.6.4 The cassette is placed in 10% buffered formalin. Use a volume of formalin that is appropriate for the number of cassettes fixed at one time.
- 7.6.5 Fixed tissues are processed in an automated tissue processor and embedded in paraffin.
- 7.7 Record data
 - 7.7.1 Data should be recorded at the time of tissue acquisition or as soon as possible thereafter.
 - 7.7.2 Data may be documented electronically at the time of acquisition or on paper and then entered into a database at a later time.
 - 7.7.3 Paper documents (biospecimen forms and consent forms) containing patient health information are stored in a locked room in a locked cabinet or scanned and saved electronically on a secure drive.
 - 7.7.4 Electronic data is secured through institutional firewalls and password protected.
 - 7.7.5 Electronic data may be entered into the ACSR database or formatted in Excel and uploaded to ACSR database at regular intervals.

8.0 APPLICABLE REFERENCES, REGULATIONS AND GUIDELINES

- 8.1 NCI Best Practices for Biospecimen Resources.
<http://biospecimnes.cancer.gov/practices/default.asp>
- 8.2 Declaration of Helsinki.
<http://www.wma.net/en/30publications/10policies/b3/index.html>
- 8.3 Best Practices for Repositories IV. Collection, Storage and Retrieval of Human Biological Materials for Research. International Society for Biological and Environmental Repositories (ISBER). Feb 2018 <http://www.isber.org/?page=BPR>

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- 8.4 US National Biospecimen Network Blueprint
<http://biospecimens.cancer.gov/resources/publications/reports/nbn.asp>
- 8.5 National Bioethics Advisory Commission: Research involving human biological materials: Ethical issues and policy guidance, Vol. I: Report and recommendations of the National Bioethics Advisory Committee. August 1999.
<http://bioethics.georgetown.edu/nbac/hbm.pdf>
- 8.6 Canadian Tumour Repository Network Standard Operating Procedures
<http://www.ctrnet.ca/operating-procedures>
- 8.7 Texas Cancer Research Biobank(<http://txcrb.org/>)
- 8.8 Preece, Ann., H.T. (ASCP, 1972. A Manual for Histologic Technicians, 3rd ed., Little Brown and Company, Boston

9.0 APPENDICES

Not Applicable.

10.0 REVISION HISTORY

| SOP Number | Date revised | Author | Summary of Revisions |
|------------|--------------|-----------|--|
| Tech002 | 3-28-2018 | AL/BGG/TY | Replace sample with biospecimen, formatting and definitions. |
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