AIDS and Cancer Specimen Resource (ACSR)	Effective Date: 06/15/2015
Technical: Solid Tissue Collection	Version 1.0
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Approved by ACSR Executive Committee	06/15/2015

1.0 PURPOSE

The purpose of this document is to establish the procedure to collect solid tissues for the AIDS and Cancer Specimen Resource (ACSR). Tissue samples are collected from patients with informed consent. Only tissues that are in excess of those required for clinical diagnosis will be collected. Tissue procured from sources other than the ACSR RBRs must be evaluated.

2.0 SCOPE

This standard operating procedure (SOP) describes how tissues should be harvested, processed and stored. This SOP applies to all personnel from ACSR Regional Biospecimen Repositories (RBRs) and affiliates that are responsible for performing tissue procurement specifically for the ACSR. The 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

4.0 ROLES AND RESPONSIBILITIES

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

ACSR Personnel	**Responsibility/Role**
Research Nurse or Research Technician	Obtain Patient Consent.
Pathology Personnel	Resection of cancer (and tissue match normal) specimen based on gross examination.
ACSR Staff Member	Transport tissue, process blood, label vials, data entry and storage.

5.0 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)**
ACSR Collection Form	The ACSR Collection Form will include patient and tissue identifiers.
Personal Protection Equipment (PPE)	Gloves, gown/scrubs, lab coat, face shield, etc. as appropriate for the environment.
Cryo marking pen, pre-labeled container, or preprinted labels.	Specimen labels might be hand written on the specimen container (such as Statmark #SMP-BK). Pre-printed labels or pre-labeled containers might be used.
Sterile specimen container	(such as VWR# 15704-085 or Fisher #14-828-320)
Clean forceps	
Clean scalpel blades	(such as Fisher #31-200-32 or VWR # 21909-654)
Aluminum foil	
Laboratory gloves	(such as VWR #82026-426 or Fisher #19-130-1597C)
Needle/sharps disposal unit	(such as Fisher #14-827-63 or VWR#19001-005)
Sterile saline (0.9% sodium chloride)	
Various cooling materials in appropriate containers. (wet ice, dry ice, liquid nitrogen and/or isopentane.)	Freezing processes vary at different sites. Each follows these guidelines 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 or isopentane. Tissues should never be frozen on dry ice.
Optimal Cutting Temperature	

Compound (OCT Tissue Tek #4583)	
Appropriate storage containers	The storage container is site specific. Specimen may be snap frozen in liquid nitrogen and stored in a cryovial, or they might be frozen in OCT and stored in a cryo-cassette (Provia PSC-1-Proviasette or cardboard pill boxes such as Argos Technologies #89085-506) wrapped in foil.
-80 freezer	

6.0 DEFINITIONS

See ACSR Glossary.

7.0 PROCEDURES

This procedure is intended to ensure that specimens obtained from consented participants are collected and 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 collecting and handling all specimens.

7.1.2 Use PPE (personal protective equipment) in accordance with collecting institution's guidelines.

7.1.3 Standard best-practice working procedures include careful manipulation of the patient samples, disinfection of countertops and equipment used during testing, and disposal of biohazard waste into appropriate receptacles.

7.2 Verification of Identification Information on Collection Vessels

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

7.3 Collection, General Considerations

7.3.1 Prepare tissue collection forms, which include: 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 and tissue collection vessels.

7.3.2 Prepare tissue collection vessels by labeling them with the patient name (initials), hospital number or surgical number, date, time. The container must also have paperwork identifying the tissue and patient disease/status.

7.3.3 After removal from the patient, the tissue must be placed in a labeled sterile container and preferably on gauze saturated with saline or phosphate buffered saline solution. Drying can create artifact in the specimen.

7.3.4 Pathology staff will determine the amount of bankable tissue.

7.3.5 The time between resection and freezing should be less than 1 hour.

7.3.6 If there will be a delay before the tissue is prepared by the pathologist or technician, place the specimen in a refrigerator or place the specimen 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 Collection, Optimal Cutting Temperature (OCT) Compound

7.4.1 Fill wide mouth dewar 1/3 full with liquid nitrogen.

7.4.2 Fill 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 -150^{0}C thermometer and keep stirring until the isopentane is quenched to -150^{0}C.

7.4.5 Remove the thermometer.

7.4.6 Trim tissue to a size no larger than 12mm^2 and 4mm thick.

7.4.7 Fill embedding mold half way with OCT compound.

7.4.8 Place tissue on top of OCT in mold, cover the tissue with layer of OCT until the mold is filled.

7.4.9 Bend up the lip of the mold and label plastic mold with block number and initials or location if more than one patient or more than one organ source.

7.4.10 Repeat until all tissue is placed in molds and covered with OCT.

7.4.11 Do not let tissue sink to the bottom of the mold.

7.4.12 Using the long 12" forceps grab the handle of the cryomold that contains the specimen 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^{0}C or in a container with dry ice until ready to store all samples.

7.4.14 Monitor the temperature of the isopentane. Warm or cool as necessary to maintain -150^{0}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 or cryo-bag. Store in -70 to -80^{0}C freezer. Samples will keep for years if stored properly.

7.5 Collection, Snap freeze in liquid nitrogen (LN_2)

7.5.1 Different procedures result in varied amount of tissue available for biobanking. If the available tumor tissue is less than 100mg (~5mm^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), to cut off a portion and return the vial for storage.

7.5.2 Matched normal tissue (50-100mg) is collected at the same time.

7.5.3 Annotate collection form and label collection vials with coded identifiers.

7.5.4 Drop vial in LN_2 for 2 minutes.

7.5.5 Transfer the vial to -80^{0}C freezer for long term storage.

7.6 Collection, Formalin Fixed Paraffin Embedded (FFPE)

7.6.1 Tumor tissue might also be collected and fixed. This tissue is used to assess the quality of the frozen specimen.

7.6.2 For any tumor specimen, 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 Place the cassette 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 Collection, Data record.

7.7.1 Data collection must be done at the time of tissue collection.

7.7.2 Data might be documented electronically at the time of collection or on paper and then entered into a database at a later time.

7.7.3 Paper documents (collection forms and consent forms) containing patient health information are stored in a locked room in a locked cabinet.

7.7.4 Electronic data is secured through institutional firewalls and password protected.

8.0 APPLICABLE REFERENCES, REGULATIONS AND GUIDELINES

8.1 NCI Best Practices for Biospecimen Resources.
http://biospecimens.cancer.gov/bestpractices/2011-NCIbestpractices.pdf

8.2 Declaration of Helsinki.
http://www.wma.net/en/30publications/10policies/b3/index.html

8.3 Best Practices for Repositories: Collection, Storage and Retrieval of Human Biological Materials for Research. International Society for Biological and Environmental Repositories (ISBER). http://c.ymcdn.com/sites/www.isber.org/resource/resmgr/Files/ISBER_Best_Practices_3rd_Edi.pdf

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

10.0 REVISION HISTORY
