AIDS and Cancer Specimen Resource

AIDS and Cancer Specimen Resource (ACSR)	Effective Date: August 2018
Technical: Live Tissue Collection	Version 2.0
ACSR SOP ID# Tech006	Page 1 of 13
Approved by ACSR Executive Committee	July 2018

1.0 PURPOSE

The purpose of this document is to establish the procedure to collect live cells from peripheral blood, bone marrow, ascites, and tissue fine needle aspirate (FNA) for the AIDS and Cancer Specimen Resource (ACSR).

2.0 SCOPE

This standard operating procedure (SOP) describes how tissue and live cells should be processed and stored. This SOP applies to all personnel from ACSR Regional Biospecimen Repositories (RBRs) and affiliates that are responsible for performing biospecimen acquisition specifically for the ACSR. The AIDS Cancer Specimen Resource Regional Biorepositories 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. 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

ACSR SOP Tech009 Biospecimen Handling

ACSR SOP Tech010 Biospecimen Labeling

4.0 DEFINITIONS

Term/Acronym	Definition
ACSR	AIDS and Cancer Specimen Resource
Aliquot	The sample has the original characteristics of the original or parent specimen but in smaller quantities (I 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.
Biospecimen	A sterile container used to transport biospecimens such as: urine

Vessel	cup, draw tube, conical tube or capped vessel.
BSL2	Bio Safety Level 2
Derivative	The original characteristics of the specimen are changed (FFPE vs DNA derived from FFPE).
EC	Executive Committee
FNA	Fine Needle Aspirate
LSM	lymphocyte separation medium
PBS	Phosphate Buffered Saline
PPE	Personal Protective Equipment such as gloves, lab coat, face shield.
RBR	Regional Biorepository
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.
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 member RBRs and affiliate sites that are responsible for collecting and processing of live cells for storage.

ACSR Personnel	**Responsibility/Role**
ACSR staff member	Process biospecimen, label vials, perform data entry and record storage.

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/Processing form	
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.
Personal protection Equipment (PPE)	Gloves, gown/scrubs, lab coat, face shield, etc. as appropriate for the environment.
Sterile biospecimen container	VWR# 15704-085 or Fisher #14-828-320
Blood draw vacutainer	Heparinized green top BD #367874 ACD (Acid Citrate Dextrose) tube BD #364816 EDTA (lavender top) tube BD# 368661
Swing bucket centrifuge	Beckman Coulter Allegra X22
Various pipettors and tips as appropriate for the volume of fluid.	Serological Pipettes: 5ml VWR# 89130-908 10 ml VWR# 89130-910 25 ml VWR# 89130-912
Laboratory gloves	VWR #82026-426 or Fisher #19-130-1597C
Ficoll hypaque or lymphocyte separation medium (LSM)	[hydrophilic polysaccharide solution with a p=1.077g/ml] GE Ficoll-Paque Plus VWR # 95038-168
15ml tubes and 50ml conical tubes	BD Falcon #350296 and # 352070
Phosphate buffered saline	PBS- Cellgro #21-040-CV
Freezing media	Freezing media is typically culture media fortified with Fetal Bovine Serum (FBS-Gemini#100-106) and 10% dimethyl sulfoxide (DMSO Sigma #D2438 or equivalent). Specialized freezing media may also be purchased (Life Technologies #12648010).

Cryovials suitable for liquid nitrogen storage	Nalgene # 5000-0012
Cell freezing containers	Various cell freezing systems might be used to slow freeze live cells. (Mr Frosty-Thermoscientific #5100-001 with isopropanol or Bioscision cool cell #BCS-136)
Biosafety cabinet (BSL2) to ensure worker safety and to maintain integrity of the biospecimen	
Hemocytometer trypan blue for viable cell counting	Sigma Bright-Line Z359629 Sigma T8154
Automated Cell Counter	Beckman Coulter Vi-Cell XR
Kim wipes	
10% bleach solution	
70% ethanol solution	
4^{0}C refrigerator	
-20^{0}C freezer	
-80^{0}C freezer	

7.0 PROCEDURES

This procedure is intended to ensure that live cell biospecimens are 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 (personal protective equipment) 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 Biospecimen Vessel

As applicable, verify the accuracy of coded patient information (in keeping with privacy and ethical policies) and ensure that it corresponds with the information on labels on biospecimen or biospecimen vessels or tubes. 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 All steps should be performed in a biosafety cabinet and using good tissue culture technique to prevent bacterial contamination.

7.3.2 Hood surfaces, pipettors and gloves should be cleaned with 70% ethanol before and after processing (Appendix 9.1).

7.3.3 The container should have coded identifier, date, time and accompanying paperwork identifying tissue and disease/status.

7.3.4 Keep cell freezing containers (with isopropanol) cooled at 4^0C.

7.3.5 Prepare biospecimen form and cryovials.

7.3.6 Prepare cell freezing media. Keep on ice until ready to use.

7.3.7 If processing blood from more than one patient, clearly label tube so that specimens are not swapped. Preferably, only one case should be processed at a time.

7.4 Ascites processing

7.4.1 Transfer ascites to 50ml centrifuge tubes.

7.4.2 Centrifuge ascites fluid for 10 minutes @ 250-400xg at 4^oC.

7.4.3 Annotate processing form and label storage vials with coded identifiers.

7.4.4 Using a pipet, take up 5ml of cleared ascites fluid.

7.4.5 Aliquot into cryovials at 1ml volumes.

7.4.6 Keep on ice until ready to freeze.

7.4.7 Decant or aspirate the remaining fluid carefully. Do not disturb the cell pellet.

7.4.8 Resuspend cells in 3-5ml PBS.

7.4.9 Go to 7.7.12

7.5 Isolate Peripheral Blood Mononuclear Cells

7.5.1 Mix blood by inverting the non-coagulating draw tube and note color of draw tube on processing paperwork.

7.5.2 If SAVING plasma, add 15ml Ficoll to 50ml tubes or 6ml Ficoll to 15ml tubes. Carefully layer PB+PBS mixture over ficoll, using 6ml PB to 5ml ficoll ratio.

7.5.3 If NOT SAVING plasma, dilute blood in a 50ml tube with and equal volume of PBS. Add 15ml Ficoll to 50ml tubes or 6ml Ficoll to 15ml tubes. Carefully layer PB over ficoll, using 6ml PB to 5ml ficoll ratio.

7.5.4 Use as many tubes as needed to process all the blood.

7.5.5 Go to 7.7.2.

7.6 Isolate Mononuclear Cells from Bone Marrow aspirate

7.6.1 Bone Marrow biospecimens should come in non-coagulating collection draw tubes. Note color of draw tube on processing paperwork.

7.6.2 Add 6ml Ficoll to 15ml tubes. Carefully layer up to 6ml of Bone Marrow on top. Use as many tubes as needed to process the biospecimen(s).

7.6.3 Go to 7.7.2.

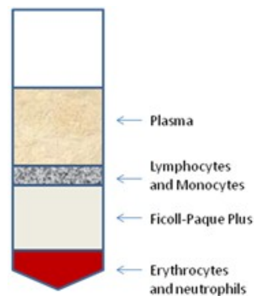
7.7 Isolate cells from Fine Needle Aspirate

7.7.1 If live cells are to be banked from FNA procedure, homogenize tissue in PBS or media. Add 6ml Ficoll to 15ml tubes. Carefully layer up to 6ml of the FNA biospecimen on top. Use as many tubes as needed to process the entire biospecimen. Go to 7.7.2.

7.7.2 Centrifuge for 20 minutes @ 800-900xg WITHOUT the brake.

7.7.3 Annotate processing form and label storage vials with coded identifiers.

7.7.4 Carefully handle tubes after centrifugation. Layers should be visible as



shown in the figure.

7.7.5 If SAVING plasma, remove with a pipet to a new tube, being careful not to disturb the lymphocyte/monocyte:Ficoll interface.

7.7.6 Collect lymphocyte/monocyte cell layer by pipetting and mix with the equal volume or twice volume of PBS.

7.7.7 Invert the tubes to mix and centrifuge 10 minutes @ 250-400xg.

7.7.8 Promptly decant the PBS and resuspend each cell pellet in 3ml PBS.

7.7.9 Pool all cells together and wash with 2X volume of PBS.

7.7.10 Centrifuge the cells for 10 minutes @ 250-400xg.

7.7.11 Promptly aspirate the PBS and resuspend each cell pellet in 3-5ml PBS. The volume will depend on the size of the cell pellet. If the cells are clumping together increase the volume of PBS.

7.7.12 Place cells on ice. Keep cells cool until freezing.

7.7.13 Take a sample of resuspended cells to count on a hemocytometer (Appendix 9.2).

7.7.14 Centrifuge the cells for 10 minutes @ 250-400xg.

7.7.15 Calculate the volume of freezing media needed to freeze cells @ 10-$50x10^6$ cells/ml.

7.7.16 Aspirate the PBS from the cell pellet, agitate cell pellet and slowly add freezing media while tube is on ice. Resuspend cells in freezing media. If cells are not to be recovered for culture, dry cell pellets can be frozen in liquid nitrogen and transferred to -80^0C freezer.

7.7.17 Aliquot into cryovials at 1ml volumes and label appropriately.

7.7.18 Place vials in freezing containers and transfer them to -80^0C freezer.

7.7.19 Slow-freeze overnight and transfer to liquid nitrogen for long term storage. Record storage location.

7.8 Record data

7.8.1 Data should be recorded at the time of biospecimen acquisition or as soon as possible thereafter.

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

7.8.3 Paper documents (biospecimen forms) containing patient health information are stored in a locked room in a locked cabinet or scanned and saved electronically on a secure drive.

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

7.8.5 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://biospecimens.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

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 Blood Collection: Routine Venipuncture and Specimen Handling.
http://library.med.utah.edu/WebPath/TUTORIAL/PHLEB/PHLEB.html

8.7 Preece, Ann, H.T. (ASCP), 1972. A Manual for Histologic Technicians, 3rd ed., Little Brown and Company, Boston.

9.0 APPENDICES

9.1 BSL2 HOOD CLEANING

1.0 Purpose:

The purpose of this procedure is to provide guidelines for a uniform and reproducible method for proper cleaning of the BSL2 hood before using.

2.0 Materials:

a. Laboratory gloves

b. 70% Ethanol

c. Large wipes (Kimberly Clark #34705)

d. Amphyl Disinfectant- Deodorant Spray (Reckitt Benckiser #0062231 http://www.rbnainfo.com)

3.0. Procedures:

a. Put gloves on. You may change gloves as needed.

b. Materials not needed in the hood should be removed and put away.

c. Spray the hood with 70% ethanol and wipe the inner surfaces with a wipe (especially the pan) with a solution of 70% ethanol and allow the area to dry.

d. Dispose of the used wipe in the biohazard trash.

e. Spray the intake grill with Amphyl all across the front of the hood.

f. Close the sash.

g. Remove your gloves and place them in the biohazard trash.

h. Wash your hands.

9.2 MANUAL CELL COUNTING FOR VIABILITY WITH HEMACYTOMETER

1.0 Purpose:

The purpose of this procedure is to provide guidelines for a uniform and reproducible method for counting live cells from culture or patient specimen.

2.0 Materials:

a. Trypan blue

b. Hemacytometer

c. Pipette-man

d. Pipette tips

e. 70% ethanol

f. Kimwipes

g. Calculator

3.0 Procedures:

a. Count the cells using a 1:20 or 1:400 dilution of stock Trypan Blue. (1:20 Trypan blue with PBS). Before using the 95/5 Trypan Blue, vortex it for a couple of seconds. Get the 200ul pipette-man and a 200ul pipette tip and measure out 190ul of solution and put in the micro centrifuge tube.

b. If the cells have been sitting, you should make sure that the lid to the biospecimen is secured and vortex the biospecimen a so that the cells are evenly distributed in the PBS. Use the 10ul pipette-man and pipette tip and measure out 10ul of the biospecimen and put it into the micro centrifuge tube containing the 190ul of 95/5 Trypan Blue. Secure the lid and vortex the counting tube sample for about 3 seconds.

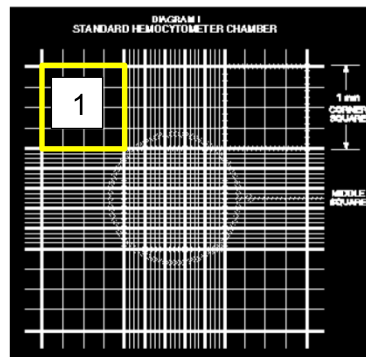
c. This is the sample you will use to count the cells. If the cells are exposed for an extended period of time, viable cells, as well as nonviable cells may begin to take up the dye.

d. Count the cells using a Hemacytometer to determine total cell counts and viability.

e. Clean the hemacytometer using 70% alcohol and a lint free kim-wipe. Clean the cover slip as well.

f. Put the cover slip on the hemacytometer. Use a 0-20ul Pipetman to transfer 10ul of the sample to both chambers of the hemacytometer. Carefully touch the edge of the cover-slip with the pipette tip and allow each chamber to fill by capillary action. Do not overfill or under fill the chambers.

g. Starting with chamber 1 count the cells in the four 1mm corner squares of the hemacytometer. Keep a separate count for the viable and non-viable cells.



h. Tally the Viable cell number of cells / number of corners counted (if both sides of the hemacytometer are counted then this is equal 8). The number of cells per corner is multiplied by 20 (the dilution factor) multiplied by 10,000 (hemacytometer dilution).

i. Example 82 viable cells / 8 corners counted x 20 dilution factor x 10,000 hemacytometer factor = 2.05 x 10^6 cell per milliliter. Total number of milliliters x cells per milliliter = total cells. Example 2.05x10^6 cells per mL x 5mL = 10.25 x 10^6

j. Percent of viable cells = live cells / (live + dead) cells = % viable.
Example 114 / 114+16 x 100% = 88% viable.

10.0 REVISION HISTORY

SOP Number	Date revised	Author	Summary of Revisions
Tech006	Mar 8, 2018	BGG/TY	Formatting, add definitions and replace sample with biospecimen