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## 1.0 PURPOSE

The purpose of this document is to establish the procedure to collect live cells from peripheral blood, bone marrow, ascites, and fine needle aspirate for the AIDS and Cancer Specimen Resource (ACSR). Tissue and live cell samples are collected from patients with informed consent. Only tissues that are in excess of those required for clinical diagnosis will be collected.

## 2.0 SCOPE

This standard operating procedure (SOP) describes how tissue and live cells 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

ACSR SOP ID# Tech009 Specimen Handling

## 4.0 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 and tissue for storage.

ACSR Personnel	Responsibility/Role
Research Nurse or Research Technician or Equivalent	Obtain Patient Consent.
Clinic staff or other qualified personnel	Assists with collection of aspirates and blood.
ACSR staff member	Transport and process specimen, label vials, data entry and storage.

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## 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 Specimen Collection form	The ACSR Collection Form will include patient and tissue identifiers.
Cryo marking pen, pre-labeled container, or preprinted labels.	Specimen labels might be hand written on the specimen container (Statmark #SMP-BK). Pre-printed labels or pre-labeled containers might be used.
Personal protection Equipment (PPE)	Gloves, gown/scrubs, lab coat, face shield, etc. as appropriate for the environment.
Sterile collection container	For specimen other than blood. (VWR# 15704-085 or Fisher #14-828-320)
Blood collection 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 or lymphocyte separation medium (LSM)	[hydrophilic polysaccharide solution with a $\rho=1.077\text{g/ml}$ ]
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 90% Fetal Bovine Serum (FBS-Gemini#100-106 or lot

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	tested equivalent) and 10% dimethyl sulfoxide (DMSO Sigma #D2438 or equivalent). Specialized freezing media might also be purchased (Life Technologies Recovery Cell Culture Freezing Media 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- Thermo Scientific #5100-001 or Bioscience cool cell #BCS-136) and isopropanol.
Biosafety cabinet (BSL2) to ensure worker safety and to maintain integrity of the sample	
Hemocytometer trypan blue for cell counting.	Sigma Bright-Line Z359629 Sigma T8154
Automated Cell Counter	Beckman Coulter Vi-Cell XR
4°C refrigerator	
-20°C freezer	
-80°C freezer	

## 6.0 DEFINITIONS

See ACSR Glossary.

## 7.0 PROCEDURES

This procedure is intended to ensure that live cell samples 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.

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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 Identifying Information

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 collection tubes. Ensure that all personnel are trained in the use of the 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 cells (Appendix 9.1).

7.3.3 The container must have patient identifiers (name or initials, hospital number, or surgical number) and must be accompanied by paperwork identifying tissue and patient disease/status, date and time of collection.

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

7.3.5 Prepare collection 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 tubes so that specimens are not swapped. Preferably, only one case should be processed at a time.

## 7.4 Ascites

7.4.1 Transfer ascites to 50ml centrifuge tubes.

7.4.2 Centrifuge ascites fluid for 10 minutes @ 250-400xg at 4°C.

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

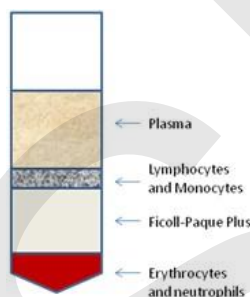
7.4.4 Collect 5ml of cleared ascites fluid.

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- 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 Peripheral Blood Mononuclear Cells from blood.
  - 7.5.1 Mix blood by inverting the non-coagulating collection tube.
  - 7.5.2 Dilute blood in a 50ml tube with an equal volume of PBS.
  - 7.5.3 Add 15ml Ficoll to 50ml tubes or 6ml Ficoll to 15ml tubes. Carefully layer an equal volume of diluted blood on top. Use as many tubes as needed to process all the blood.
  - 7.5.4 Go to 7.7.1
  
- 7.6 Bone Marrow aspirate.
  - 7.6.1 Bone Marrow samples should come in non-coagulating collection tubes. If the sample is in a different color topped tube, make sure to indicate this on the 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 entire sample.
  - 7.6.3 Go to 7.7.1
  
- 7.7 Fine needle aspirate (FNA).
  - If live cells are collected from FNA procedure, add 6ml Ficoll to 15ml tubes. Carefully layer up to 6ml of the FNA sample on top. Use as many tubes as needed to process the entire sample. Go to 7.7.1
  - 7.7.1 Centrifuge for 20 minutes @ 800-900xg WITHOUT the brake.
  - 7.7.2 Annotate collection form and label collection vials with coded identifiers.
  - 7.7.3 Add 20ml PBS to new 50ml tubes in the hood.

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7.7.4 Carefully handle tubes after centrifugation. Layers should be visible as shown in the figure.



- 7.7.5 Collect lymphocyte/monocyte cell layer by pipetting and mix with the PBS that was prepared in 7.7.3
- 7.7.6 Invert the tubes to mix and centrifuge 10 minutes @ 250-400xg.
- 7.7.7 Promptly decant the PBS and resuspend each cell pellet in 3ml PBS.
- 7.7.8 Pool all cells together and bring the volume up to 40ml with PBS.
- 7.7.9 Centrifuge the cells for 10 minutes @ 250-400xg.
- 7.7.10 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.
- 7.7.11 Place cells on ice. Keep cells cool until freezing.
- 7.7.12 Take a sample of resuspended cells to count on a hemocytometer (Appendix 9.2).
- 7.7.13 Centrifuge the cells for 10 minutes @ 250-400xg.
- 7.7.14 Calculate the volume of freezing media needed to freeze cells @ 10-50x10<sup>6</sup> cells/ml.
- 7.7.15 Aspirate the PBS from the cell pellet and 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°C freezer.
- 7.7.16 Aliquot into cryovials at 1ml volumes.
- 7.7.17 Place vials in freezing containers and transfer them to -80°C freezer.
- 7.7.18 Freeze overnight and transfer to liquid nitrogen for long term storage.

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7.8 Collection, Data record.

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

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

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

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

7.8.5 Electronic data should be formatted in excel and uploaded to the ACSR database at regular intervals.

## 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](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 Blood Collection: Routine Venipuncture and Specimen Handling.

<http://library.med.utah.edu/WebPath/TUTORIAL/PHLEB/PHLEB.html>



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

## 9.0 APPENDICES

### 9.1 BIOREPOSITORY BSL2 HOOD CLEANING

#### Hood Cleaning

##### 1.0. Purpose:

To provide guidelines for a uniform and reproducible method for proper cleaning of the hood before using.

##### 2.0. Materials:

- 2.1 Latex exam gloves
- 2.2 70% Ethanol
- 2.3 Large wipes (Kimberly Clark #34705)
- 2.4 Amphyl Disinfectant- Deodorant Spray (Reckitt Benckiser #0062231  
<http://www.rbnainfo.com>)

##### 3.0. Procedures:

- 3.1. Put gloves on. You may change gloves as needed.
- 3.2. Materials not needed in the hood should be removed and put away.
- 3.3. 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.
- 3.4. Throw the used wipe in the biohazard trash.
- 3.5. Spray the intake grill with Amphyl all across the front of the hood.
- 3.6. Close the sash and turn off the u.v. light.
- 3.7. Remove your gloves and out then in the biohazard trash.
- 3.8. Wash your hands.



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## 9.2 BIOREPOSITORY MANUAL CELL COUNTING

### Manual Cell counting

#### 1.0. Purpose:

To provide guidelines for a uniform and reproducible method for counting live cells from culture or patient specimen.

#### 2.0. Materials:

- 2.1 Trypan blue
- 2.2 Hemacytometer
- 2.3 Pipette-man
- 2.4 Pipette tips
- 2.5 70% ethanol
- 2.6 Kimwipes
- 2.7 Calculator

#### 3.0. Procedures:

- 3.1. 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.
- 3.2. If the cells have been sitting, you should make sure that the lid to the sample is secured and vortex the sample so that the cells are evenly distributed in the PBS. Use the 10ul pipette-man and pipette tip and measure out 10ul of the sample and put it into the micro centrifuge tube containing the 190ul of 95/5 Trypan Blue. Secure the lid and vortex the sample for about 3 seconds.
- 3.3. 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.
- 3.4. Count the cells using a Hemacytometer to determine total cell counts and viability.
- 3.5. Clean the hemacytometer using 70% alcohol and a lint free kim-wipe. Clean the cover slip as well.

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- 3.6. 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.
- 3.7. 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.
- 3.8. Calculate the total number of cells. Viable cell number/number of corners counted (if both sides of the hemacytometer are counted then this is equal 8).
- 3.9. Then that number is multiplied by 20 (the dilution factor) multiplied by 10,000 (hemacytometer dilution). Example  $82 / 8 \times 20 \times 10,000 = 2.05 \times 10^6$  cell per sample. Total number of mls x cells per ml = total cells.
- 3.10. Percent of viable cells = live cells / live and dead cells = % viable. Example  $114 / 114+16 \times 100\% = 88\%$  viable.

## 10.0 REVISION HISTORY

SOP Number	Date revised	Author	Summary of Revisions