University of Arizona Cancer Center
Room 0917 Phone (520) 626-7319
UACC-TACMASS@uacc.arizona.edu

SOP #1 | Subject **Bake and Dewax** | Sheet **1** of **2**

Rev 1		Effective Date	Author
1: 7/1/2016		7/1/2016	Fimbres

1. PURPOSE

The purpose of this protocol is to prepare slides for staining with aqueous stains.

2. SCOPE

This format applied to all SOP's developed within the TACMASR laboratory.

3. RESPONSIBILITIES

This SOP can be carried out by someone who has laboratory certifications and has been through TACMASR training.

4. MATERIALS and EQUIPMENT

70% ethanol
95% ethanol
2x 100% ethanol
3x xylene
Gloves
Leica autostainer with program to bake and deparaffinize
Leica 30-slide rack

5. SAFETY AND CAUTIONARY NOTES

Attached

6. PROCEDURE

6.1 Load slides onto rack.
6.2 Load slides onto Leica autostainer slot "LOAD."
6.3 Begin program 1 (pre-programed to bake and dewax)
6.3.1 Program 1:
6.3.2 Bake slides for 20 minutes in baking slot.
6.3.3 Xylene 1 for 3 minutes
6.3.4 Xylene 2 for 3 minutes
6.3.5 Xylene 3 for 3 minutes
6.3.6 Ethanol for 3 minutes
6.3.7 Ethanol for 3 minutes
6.3.8 95% ethanol for 3 minutes
6.3.9 70% ethanol for 3 minutes
6.3.10 Program 1 calls for water rinse for 1 minute but can be removed from the program for hand staining.

7. Results

Slides should be hydrated and ready to be stained according to staining protocol.

University of Arizona Cancer Center
PROCEDURE
Room 0915/0917 Phone (520)626-7319
UACC-TACMASS@uacc.arizona.edu

SOP #

Brady System Cassette labeling

Rev 0		Effective Date	Author
3/29/2016		3/29/2013	Kepler

1. PURPOSE

The purpose of this Standard Operating Procedure (SOP) is to outline the process for labeling tissue cassettes using the Brady BBP™11-34L Thermal Transfer Printer and BSP™31 Label Attachment. This system provides accurate and legible labels for tissue cassettes that remain permanently attached throughout the entire sample management process.

2. SCOPE

This SOP applies to all tissue cassettes that are to be labeled within the Tissue Acquisition and Cellular/Molecular Analysis Shared Resources within the Arizona Cancer Center. Writing on cassettes by hand using a solvent resistant marker is often hard to read and can wash off or become smudged during the processing leading to errors.

3. REFERENCE DOCUMENTS

3.1 Brady BBP™11-34L Thermal Transfer Printer User Guide
3.2 BSP™31 Label Attachment System User Guide

4. RESPONSIBILITIES

4.1 Laboratory personnel using the Brandy™11-34L Thermal Transfer Printer and BSP™31 Label Attachment are responsible for following the procedures outlined in the SOP.

5. MATERIALS and EQUIPMENT

5.1 Brady BBP™11-34L Thermal Transfer Printer
5.2 BSP™31 Label Attachment System
5.3 Compatible PC
5.4 Thermal Transfer Printer Ribbon
5.5 Filter Kit
5.6 Probe Cleaning Kit

6. SAFETY AND CAUTIONARY NOTES

6.1 Gloves should be worn when changing out the filter unit

7. PROCEDURE

7.1 Turn on the computer, the password is Tacmass#3959
7.2 Open the program “CODESOFT 8 PRO”
7.3 Pull down the “File” menu and choose CassetteLabel Temp-BradyBBP CK

7.4 There will be two vertical lines on the screen. Take care to write within the lines or else the writing will exceed the length of the label. Two horizontal lines can fit on the label.

7.5 On the top of the label write the PI's identification. On the bottom horizontal line write the PI name, several spaces and the TACMASR Project number (T number)

7.6 Under file, select Print printer, BBP™34. Then select print and print out the label.

7.7 Remove the printed label from the backing sheet and attach it to a cassette. Gently rub your finger across the label to make sure that it adheres to the cassette.

7.8 Turn on the BSP™31 Label Attachment System by pressing the "POWER" button on the front of the instrument. The 3 yellow status lights will blink while the instrument is warming up. When the lights are steady green the instrument is ready. Normal warm up time is 3 minutes.

7.9 Insert and immediately release the cassette label side up and facing into the unit. The stats lights will flash green and the riveting process will begin.

7.10 When the riveting process is complete the status lights will turn a steady green and the cassette can be removed. Continue in the same manner with any remaining cassettes to be riveted.

7.11 After all labels have been attached to cassettes, press the "POWER" button on the front of the instrument. This initiates an auto clean and cool down cycle.

7.12 During normal usage the probes accumulate residue. A probe cleaning brush is provided to clean the probes. Unit should be turned off. Press and hold the power button and simultaneously turn the main power switch on. This puts the instrument into probe cleaning mode and the status lights will flash alternating yellow and green.

7.13 Use a flashlight to identify the probes, insert the brush and gently brush up and down.

7.14 Turn the main power switch to off when finished.

7.15 The filter should be replaced after approximately 5000 uses. Around 4200 cycle the filter light will turn yellow indicating that the filter needs to be replaced. The unit will not work without a filter. Detailed instructions for changing the ribbon, media, filter and cleaning the probes can be found in the user guide along with a troubleshooting section.

8. **RECORDS**

8.1 Record the date and initial on the following log sheet when the ribbon, media or filter is replaced or when the probes are cleaned.

8.2 Make a notation when there are any maintenance issues or if the instruments malfunction.

and the BSP™31 Label Attachment System Maintenance Log

DATE	ACTION TAKEN	INITIALS

1. The filter should be replaced after approximately 5000 uses. Around 4200 cycles the Filter light will turn yellow indicating that the filter needs to be replaced. The unit will not work without a filter.
2. Detailed instructions for changing the ribbon, media, filter and cleaning the probes can be found in the user guide along with a troubleshooting section.

University of Arizona Cancer Center
PROCEDURE
Room 0915/0917 Phone (520)626-7319
UACC-TACMASS@uacc.arizona.edu

SOP #

Cell Pellet Preparation

Rev 0		Effective Date	Author
3/28/2016		3/28/2016	Kepler

1. PURPOSE

The purpose of this standard operating procedure (SOP) is to establish the format for preparing cell blocks of cultured cells.

2. SCOPE

This standard operating procedure applies to all cell lines brought to the Tissue and Cellular/Molecular Analysis Shared Resources within the Arizona Cancer Center.

3. REFERENCE DOCUMENTS

3.1 Thermoscientific Shandon Cytoblock Kit Ref. 7401150
3.2 TACMASR worksheet

4. RESPONSIBILITIES

4.1 Laboratory personnel making cell blocks are responsible for following the procedures outlined in this SOP and notifying the lab manager when deviations or unexpected events arise.
4.2 Laboratory personnel making the cell blocks are responsible for filling out the TACMASR worksheet upon completion.
4.3 Laboratory manager is responsible for all completed projects.

5. MATERIALS and EQUIPMENT

5.1 Thermoscientific Shandon Cytoblock Kit Ref 7401150
5.2 Centrifuge, Sorvall
5.3 Vortex
5.4 Cotton Q-tip applicatiors
5.5 70% Ethanol
5.6 Tissue-Tek marking pencil

6. SAFETY AND CAUTIONARY NOTES

6.1 Gloves and laboratory coat should be worn while performing this procedure.
6.2 Formalin should be discarded in the chemical waste container located in room 0917.

7. PROCEDURE

7.1 Tissue culture cells should be harvested and fixed in formalin for 1 hour at room temperature or over night at 4°C by the investigator prior to bringing them to TAMASR. Ideally cells should be in a 15ml centrifuge tube labeled with the investigators ID.

7.2 Using a Tissue-Tek marking pencil, label the tissue cassette provided in the cell block with the investigators sample ID.

7.3 Gently vortex the 15ml centrifuge tube containing the cells to resuspend the cells. Sometimes cells are so pelleted that after centrifugation it becomes impossible to thoroughly mix the clotting reagents with all the cells if this step is omitted.

7.4 Centrifuge the tube for 3 minutes @ 1500rpm, (setting #3) in the Sorvall LegenRT centrifuge located in room 0915.

7.5 Pour off and discard the formalin, making sure to get all of the formalin off but do not disturb the cell pellet. Use a Q-tip to soak up any remaining drops on the edge of the tube.

7.6 Depending on the size of the cell pellet, while gently vortexing the tube, add 3-4 drops of reagent #2 (blue color dropper). A large pellet will require 4 drops.

7.7 Continue to vortex and add an equal amount of reagent #1, 3-4 drops (clear color dropper). A gel should immediately form.

7.8 Using the wooden end of the Q-tip, transfer the gel pellet to the circumference of the well in labeled tissue cassette.

7.9 Close the cassette and place in 70% ethanol until processing.

7.10 Cassettes are processed on the standard tissue processor program.

University of Arizona Cancer Center
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UACC-TACMASS@uacc.arizona.edu

TACMASR OPERATING PROCEDURE

SOP #0	Subject	Sheet 1 of 1

Rev 0		Effective Date	Author
	Collecting Organoids and placing into Histogel Protocol	06/12/2020	BW

1. **PURPOSE: To collect, store and embed organoids**

2. **SCOPE: Collect, embed or long-term storage of organoids**

3. **RESPONSIBILITIES: Properly collect, store and embed organoids**

4. **MATERIALS and EQUIPMENT: Organoids, pipette, cold PBS, tubes, centrifuge, paraformaldehyde, Histogel, ice, PPE**

5. **PROCEDURE:**
   - **#1. Collect attached/desired cells by removing media and covering in cold PBS to dissolve Matrigel. Harvest cells and PBS into tube(s), repeat process swishing around the well to ensure all cells have been collected.**
   - **#2. Centrifuge tube(s) for 5 minutes @ 400 g (1200 RPM's)**
   - **#3. Remove PBS from tube(s) carefully, leaving organoids that have collected near bottom of tube(s). Leaving a tiny bit of PBS is OK.**
   - **#4. Fix in 3.7% Paraformaldehyde (room Temp) for 20 minutes.**
   - **#5. Centrifuge tube(s) for 5 minutes @ 400 g (1200 RPM's)**
   - **#6. Remove Paraformaldehyde then add 200-300 ml Histogel, suspend cells throughout the Histogel by gently sucking them up and releasing them throughout the gel a few times. Place on ice for at least 20 minutes or until the Histogel is set and no longer in a liquid state. (I have left on ice in the refrigerator overnight).**
   - **#7. Long term storage can be done by harvesting, fixing and storing in PBS in refrigerator. The sample should be centrifuged after storage and before removing PBS.**
   - **#8. Send to lab on ice for process and embedding**
6. **Safety and Cautionary Notes:**
   Once melted histogel should stay at approximately 60-degrees Celsius. It will degrade with time and evaporate. To the best of our Knowledge smaller amounts of Histogel can be removed from tube and melted for use thus saving the rest in the refrigerator for future use

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UACC-TACMASS@uacc.arizona.edu

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UACC-TACMASS@uacc.arizona.edu

TACMASR OPERATING PROCEDURE

SOP #1 | Subject **Decalcification of Tissue up to Sectioning** | Sheet **1** of **1**

Rev 1		Effective Date	Author
9/13/17		9/13/17	Fimbres

1. PURPOSE

1.1 The purpose of this protocol is to decalcify tissue as to make it usable for microtomy.

2. SCOPE

2.1 This SOP can be used on bone sections and calcified arteries submitted to TACMASR.

3. RESPONSIBILITIES

3.1 The person using these materials must be aware of the lab's rules and regulations and use safety precautions when necessary.

4. MATERIALS and EQUIPMENT

4.1 Tissue to decalcify
4.2 10% formalin
4.3 Running distilled water source
4.4 Formical-2000 (Formic acid, EDTA, decalcifier) – American Master Tech, Item # CDF20QT
4.5 (Microtome and associated sectioning tools)

5. SAFETY AND CAUTIONARY NOTES

5.1 Gloves should be worn when handling formalin and Formical-2000.
5.2 Prior knowledge of tissue decalcifying times expected

6. PROCEDURE

6.1 Fix section in 10% buffered formalin.
6.2 Wash section in running deionized water for at least 3 minutes
6.3 Immerse rinsed section in Formical-2000 for 2-6 hours depending on calcification of section
6.4 Wash section in running deionized water for at least 3 minutes
6.5 Sectioning may be handled as non-decalcified tissue for processing, embedding and sectioning.
6.6 If chipping occurs on microtome, paraffin embedded sections may be further decalcified by placing block face down in a dish of Formical-2000, on ice, for 10 minutes.
6.6.1 Rinse block and place on ice block to continue sectioning.
6.6.2 Repeat if necessary

University of Arizona Cancer Center
Room 0917 Phone (520) 626-7319
UACC-TACMASS@uacc.arizona.edu

TACMASR OPERATING PROCEDURE

SOP # **1** Subject **Embedding of Formalin Fixed Tissue** Sheet **1** of **2**

Rev 5/9/18		Effective Date	Author
Date 2/1/15		2/1/15	Fimbres

1. PURPOSE

1.1 The purpose of this SOP is to maximize consistency in tissue embedding projects.

2. SCOPE

2.1 This SOP applies to all tissue submitted for embedding. Special embedding may be requested in which case the embedder is to make sure the request is fully understood.

3. REFERENCE DOCUMENTS

3.1 TACMASR Request Form
3.2 "Histotechnology." Frieda L Carson. 1997
3.3 "Histotechnology." Carson & Hladik. 2009

4. RESPONSIBILITIES

4.1 The person embedding the tissue should have experience with the tissue embedding instrument, its troubleshooting technques and a thorough understanding of the project's needs. Tissue orientation should be clear to the embedder as to create sections that show proper cellular components.

5. MATERIALS and EQUIPMENT

5.1 Embedding station: heated ovens, heated paraffin pool, cold plates.
5.2 Melted paraffin
5.3 Tissue molds
5.4 Tissue
5.5 Paratrimmer
5.6 Forceps
5.7 Waste
5.8 Metal spatula

6. SAFETY AND CAUTIONARY NOTES

6.1 Gloves should be worn at all times
6.2 Proper attire should be worn as to avoid paraffin contamination

7. PROCEDURE

7.1 After processing, tissue should be placed into the oven.
7.2 Remove the top of the tissue cassette to reveal processed tissue
7.3 Discard the cover of the tissue cassette to leave the cassette boat

7.4 Select a mold appropriate to the tissue size

7.4.1 The tissue should be in the middle of mold and should have at least a ¼ inch of space to the edge of the mold.

7.5 Orient the tissue

7.5.1 Spleens must be cut in half and stood on edge

7.5.2 Skin must be placed on edge

7.5.3 The flattest side of the tissue must go down on the mold

7.5.4 Sides exhibiting tumor-like morphology should go down on the mold

7.5.5 Tissue will be placed on a diagonal angle as to create less friction for microtomy use.

7.6 Bring the tissue to the first cold plate to secure the tissue to the bottom of the mold

7.7 Without touching the heated areas of the embedder, place the tissue cassette boat over the mold and add more paraffin to the top of the mold-cassette.

7.8 Take the mold-cassette contraption to the second cold plate.

7.9 Allow the paraffin to harden for ~60 minutes

7.10 Remove the paraffin block for the metal mold by using the metal spatula as a lever on the edge of the mold-cassette.

7.11 Allow the paraffin block to sit for 15 minutes to come to room temperature.

7.12 Using the paratrimmer, clean off the edges of the paraffin block.

7.13 Pair the block to the proper request form.

University of Arizona Cancer Center
Room 0917 Phone (520) 626-7319
UACC-TACMASS@uacc.arizona.edu

TACMASR OPERATING PROCEDURE

SOP # **1** Subject **Frozen sections** Sheet **1** of **2**

Rev 4/19/18		Effective Date	Author
Date 3/15/17		3/15/17	Jocelyn Fimbres

1. PURPOSE
   1.1 To produce a tissue section from fresh tissue as close to the living state as possible without loss of protein and nucleic acid targets.

2. SCOPE
   2.1 This procedure can be performed by a person trained in cryotomy or by anyone under the direct supervision of a histotechnician in this lab.

3. RESPONSIBILITIES

   3.1 It is the responsibility of the histology lab to make sure that the cryostat is set at the proper temperature for doing frozen sections and that the cryostat is clean and well maintained.

4. MATERIALS and EQUIPMENT
   4.1 Cryostat
   4.2 Specimen stages
   4.3 Disposable microtome blades
   4.4 Microscope slides
   4.5 OCT
   4.6 Brushes
   4.7 Forceps
   4.8 Gauze
   4.9 Heat dissipater

5. PROCEDURE

   5.1 Place a small amount of Oct on a specimen stage which is at room temperature
   5.2 Orient the fresh tissue in the OCT on the stage
   5.3 Place the specimen stage with tissue on one of the deep freeze stations in the cryochamber. The tissue will freeze in a few minutes. Place the heat dissipater over the tissue.
   5.4 When tissue is frozen, place the specimen onto the clamping device
   5.5 Trim the specimen sufficiently to get the entirety of the specimen face.
   5.6 Obtain a section with the forceps and brush
   5.7 Mount onto slides and place onto cold rack

5.7.1 Fix in acetone or formalin if staining procedure calls for it

5.8 Remove the specimen from the clamp

5.9 Seal the surface of the trimmed tissue with more OCT and freeze

5.10 Place tissue in -80 freezer.

University of Arizona Cancer Center
Room 0917 Phone (520) 626-7319
UACC-TACMASS@uacc.arizona.edu

SOP #1 | Subject **Gomori's Trichrome Blue Collagen** | Sheet **1** of **2**

Rev 2		Effective Date	Author
1: 10/23/17 2: 9/13/18		10/23/17 **9/13/18**	Fimbres

1. PURPOSE

The purpose of a Gomori's trichrome stain is to study the diseases of connective tissue and muscle associated with fibrotic and dystrophic changes. We expect results of blue collagen, red cytoplasm and muscle fibers and blue-to-black nuclei.

2. SCOPE

This format applied to all SOP's developed within the TACMASR laboratory

3. REFERENCE DOCUMENTS

None

4. RESPONSIBILITIES

This SOP can be carried out by someone who has seen it done before and has knowledge on the results of the tissue colors.

5. MATERIALS and EQUIPMENT

Bouin's solution (Sigma Aldrich ref# HT10132)
Weigert's Hematoxylin parts A & B (Newcomer Supply ref#1409B)
Gomori's trichrome blue collagen (ThermoFisher Scientific ref#88030)
Or Gomori's trichrome green collagen (Fisher: ES9000)
Oven
Running distilled water
Running tap water
70% ethanol
95% ethanol
2x 100% ethanol
3x xylene
Coverslips
Mounting media
Gloves

6. SAFETY AND CAUTIONARY NOTES

Attached

7. PROCEDURE

7.1 Bake slides for 15 minutes at 60-65 °C
7.2 Deparaffinize slides and hydrate to distilled water

7.3 Place slides in Bouin's for 1 minute at 60°C

7.3.1 Bouin's solution at 60C.

7.4 Wash in distilled water until yellow is removed from tissue

7.5 Place slides in *fresh* 1:1 part A:part B Weigert's Hematoxylin for 5 minutes at room temperature.

7.5.1 It is essential that Weigert's hematoxylin be fresh given that the alcoholic part evaporates quickly.

7.5.2 Make ONLY enough for the slides to be stained.

7.6 Rinse in tap water for 30 seconds

7.7 Rinse in distilled water for 1 minute or until water is running clear

7.8 Place slides in Gomori's trichrome for 10 minutes at room temperature

7.9 Quickly rinse slides in distilled water

7.9.1 Do not over rinse as the blue fades off the tissue easily – no more than a 30 second rinse.

7.10 Dehydrate slides in increasing grades of ethanol (70%, 95%, 100%, 100%)

7.11 Clear slides with three changes of xylene

7.12 Mount and coverslip

8. Results

Blue/green collagen
Red to maroon cytoplasm and muscle fibers
Blue to black nuclei

University of Arizona Cancer Center
Room 0917 Phone (520) 626-7319
UACC-TACMASS@uacc.arizona.edu

TACMASR OPERATING PROCEDURE

SOP # **1** Subject **Handling transwells for processing & embedding** Sheet **1** of **1**

Rev		Effective Date	Author
Date 1//1/2017		1/1/2017	JF

1. PURPOSE

The purpose of this protocol is to effectively process and embed transwells for future staining without damaging the thin cell layer growing on them.

2. SCOPE

This SOP applies to all transwells brought to TACMASR that need to be processed, embedded, cut and/or stained.

3. REFERENCE DOCUMENTS

"Preparation of Costar Transwell Inserts for Histology"

4. RESPONSIBILITIES

Laboratory personnel that is familiar with this protocol, has watched it being done, has the right qualifications should execute this protocol.

5. MATERIALS and EQUIPMENT

5.1 Pre-fixed Transwells sitting in 70% ethanol
5.2 Pipettes
5.3 Scalpel or blades
5.4 Tea bags
5.5 70% ethanol
5.6 Tissue processor
5.7 Tissue embedder

6. SAFETY AND CAUTIONARY NOTES

6.1 Gloves should be worn when handling tissue and while preparing the tissue cassettes.
6.2 Spent formalin and alcohol must be discarded in the chemical waste container in the lab.
6.3 All sharps are to be discarded in the sharps container located underneath the embedder.

7. PROCEDURE

7.1 Remove excess ethanol from transwells with pipette
7.2 Using a new blade, cut the membrane of the transwell along the edge
7.3 Place the cut membrane in a tea bag
7.4 Fold tea bag several times as to secure the membrane from slipping out during processing

7.5 Place folded tea bag in cassette and snap close
7.6 Keep in 70% ethanol until processing run
7.7 Processing run: TACMASR delayed start
7.8 Carefully remove membrane from tea bag
7.9 Cut circular membrane in half
7.10 Stand both half parallel to each other in embedding mold
7.11 Fill with paraffin and cool on cold plate

Hematoxylin-Phyloxine-Saffron (HPS) stain

Deparaffinize slides and bring to water

1. Place slides in Bouin's solution for 1 minute
    a. Heat Bouin's solution to 60C (microwave for 30 seconds and place in 60C oven)
2. Wash Bouin's solution off with distilled water
    a. Bouin's soluton will take long to come off tissue – several minutes. Make sure the yellow stain is off the slides.
3. Place slides in hematoxylin for 2:30
4. Wash off hematoxylin for 1 minute
5. Place slides in clearview for 30 seconds
6. Wash off clearview for 30 seconds
7. Place slides in bluing solution
8. Wash off bluing solution for 30 seconds
9. Place slides in Solution A for 7 minutes
    a. 100mL distilled water and 2g of Phyloxine B
    b. Filter solution
    c. Add 3 drops of formalin for preserving solution
10. Wash off Solution A for 30 seconds
11. Dehydrate slides
12. Place slides in Solution B for 7 minutes
    a. 200mL absolute ethanol and 6g saffron
    b. Seal tightly and place in 60C over for 1-2 weeks
13. Wash Solution B off with 2 changes of 100% ethanol
14. Bring slides to xylene
15. Coverslip slides and dry

Results:

Nuclei – blue

Cytoplasm – red shads

Muscle – pink

Elasic fibers – bright red

Collagen – yellow

Notes:

1. If the saffron solution is too strong it may be diluted with absolute ethanol. Store and use at room temperature. It has a limited life of about a month and is best when freshly made. It is important that there be no water in the saffron solution and that sections be thoroughly dehydrated before it is applied. If contaminated with moisture the solution must be discarded.

University of Arizona Cancer Center
Room 0917 Phone (520) 626-7319
UACC-TACMASS@uacc.arizona.edu

TACMASR OPERATING PROCEDURE

SOP #1 | Subject **Leica ASP6025 Processing of Tissue** | Sheet **1** of **2**

Rev 5/9/18		Effective Date	Author
Date 2/1/2015		2/1/15	Fimbres

1. PURPOSE

1.1 The purpose of this SOP is to establish the guidelines to properly process tissue using the Leica ASP 6025 Tissue Processor.

2. SCOPE

2.1 This SOP applies to all tissue received by TACMASR that is to be processed unless otherwise noted.

3. REFERENCE DOCUMENTS

3.1 TACMASR Request Form
3.2 "Histotechnology." Frieda L Carson. 1997
3.3 "Histotechnology." Carson & Hladik. 2009

4. RESPONSIBILITIES

4.1 The person processing tissue should have prior knowledge of the instrument and how to troubleshoot if needed.

5. MATERIALS and EQUIPMENT

5.1 Leica ASP6025
5.2 2 70% ethanol
5.3 80% ethanol
5.4 95% ethanol
5.5 3 100% ethanol
5.6 4 xylene
5.7 Cleaning ethanol
5.8 Cleaning xylene
5.9 Processing water
5.10 Gloves
5.11 Tissue cassettes sitting in 70% ethanol
5.12 Metal processing basket
5.13 100% Acetic acid

6. SAFETY AND CAUTIONARY NOTES

6.1 Gloves should be worn when handling reagents and tissue

7. PROCEDURE

7.1 Transfer all cassettes from 70% ethanol into metal basket
7.2 Insert metal basket in to retort
7.3 Select “Delayed Start Program”
7.3.1 70% ethanol until program begins
7.3.2 70% ethanol: 15 min
7.3.3 Processing water: 1 min – 40 degrees Celsius
7.3.4 Ethanol (70%): 15 min – Room temp
7.3.5 Ethanol (80%): 30 min – 40 degrees – Pressure/Volume change through reagent duration
7.3.6 Ethanol (95%): 90 min – 40 degrees Celsius – P/V change
7.3.7 Ethanol (100%): 45 min – 40 degrees Celsius – P/V change
7.3.8 Ethanol (100%): 45 min – 40 degrees Celsius – P/V change
7.3.9 Ethanol (100%): 45 min – 40 degrees Celsius – P/V change
7.3.10 Xylene: 45 min – 40 degrees Celsius – P/V change
7.3.11 Xylene: 45 min – 40 degrees Celsius – P/V change
7.3.12 Xylene: 45 min – 40 degrees Celsius – P/V change
7.3.13 Paraffin: 45 min – 60 degrees Celsius – P/V change
7.3.14 Paraffin: 60 min – 60 degrees Celsius – P/V change
7.3.15 Paraffin: 60 min – 60 degrees Celsius – P/V change
7.4 When tissue has finished the processing run, remove tissue from retort and transfer to oven on embedding instrument
7.5 Clean the processor with the “Standard Cleaning Program”
7.6 When the cleaning is complete, carefully clean out the sensor and the stirrer with 100% acetic acid.

University of Arizona Cancer Center
Room 0917 Phone (520) 626-7319
UACC-TACMASS@uacc.arizona.edu

TACMASR OPERATING PROCEDURE

SOP #0

Subject **Cleaning & Reagent Upkeep of the Leica ASP 6025 Tissue Processor**

Rev 0		Effective Date	Author
10/27/2017		10/27/2017	Fimbres

1. **PURPOSE**
   The purpose of cleaning and upkeeping the processor is to prevent errors in the system due to mishandling.
2. **SCOPE**
   Ensure full processing of research tissue for staining and storage purposes.

3. **REFERENCE DOCUMENTS**
   User's manual

4. **RESPONSIBILITIES**
   Only a person who has shadowed the workings of the instrument can make changes alone. Any question is to be addressed and every error notification is to be noted.

5. **MATERIALS and EQUIPMENT**

   Waste buckets
   Patience amidst confusion

6. **SAFETY AND CAUTIONARY NOTES**

   Use gloves for removal of reagents as they are carcinogens and irritate the skin upon contact.

7. **PROCEDURE**

   Reagent stations:
   Drawers:
   1. Formalin/70% ethanol
   2. **Processing water – replace every 10 uses (or days).** Instrument will indicate the reagent is due for a change in the Reagent Status menu. Remove "D2" from drawer, empty processing water & refill. Select "Reagent Status" on screen. Select "Set As Empty" when removing the container and "Set as Full" when the replacement water has been put into the drawer.
   3. **"Formalin" (70% ethanol) – replace every 5 uses (or days).** Instrument will indicate the reagent is due for a change in the Reagent Status menu. Remove "D1" from drawer, empty ethanol & refill with premade 70% ethanol in TACMASR lab. Select "Reagent Status" on screen. Select "Set

As Empty" when removing the container and "Set as Full" when the replacement 70% ethanol has been put into the drawer.

4. **Xylene replacement – filled with used xylene every 15 uses**. Upon starting a new run, the instrument will notify the user that "Xylene X has reached its maximum usage and must be replaced." The instrument will ask the user to place an empty bottle into "D3" for the used xylene to go into. Upon the completion of the Processing Run, the instrument will notify the user that used xylene has been emptied into D3. You will select ok. Upon the next processing run, the instrument will notify the user that the used xylene must be removed from "D3" and a fresh xylene must be placed into "D3." Select "yes" and proceed with the bottle changes.
5. **Ethanol replacement – filled with used ethanol when the 70% ethanol reaches a 55% ethanol concentration**. (Concentration measurements are taken by the instrument at the beginning of every processing run) Upon starting a new run, the instrument will notify the user that "70% Ethanol has reached its minimal concentration usage and must be replaced." The instrument will ask the user to place an empty bottle into "D4" for the used ethanol to go into. Upon the completion of the Processing Run, the instrument will notify the user that used ethanol has been emptied into D4. You will select ok. Upon the next processing run, the instrument will notify the user that the used ethanol must be removed from "D4" and a fresh 100% ethanol must be placed into "D4." Select "yes" and proceed with the bottle changes.
    a. The 80% ethanol will replace the 70% ethanol bottle.
    b. The 95% ethanol will replace the 80% ethanol bottle.
    c. The 100% ethanol will replace the 95% ethanol bottle.
    d. The 100% ethanol will replace the adjacent ethanol bottle.
    e. The newly inserted 100% ethanol in D4 will replace the last 100% ethanol bottle.
6. **Cleaning xylene – replaced every 5 uses**. Select "Reagent Status" on screen. Select "Set As Empty" when removing the container and "Set as Full" when the replacement xylene bottle has been put into the drawer.
7. **Cleaning ethanol – replaced every 5 uses.** Select "Reagent Status" on screen. Select "Set As Empty" when removing the container and "Set as Full" when the replacement ethanol bottle has been put into the drawer.

Bottom reagents: Must be filled slightly above the 3.8L line. Anything under this line may cause an error in the processing cycle. Simplest solution but frequently and easily ignored.

8. **Paraffin – will go through 15 cycles and ask to be changed.** When prompted for a paraffin change, attached the white paraffin hose onto the indicated attachments. The other end of the hose goes into an empty waste container (like an empty paraffin container). Valves will heat up and will begin to dump into the waste through the hose. Valve heating takes several minutes. The paraffin will begin to fill into the retort. Then the paraffin from the retort will begin to empty out through the attached hose. After ~1 liter of paraffin, there will be a pause in . Switch containers in this moment (as the fist will likely be full) and wait for the second to continue to fill. The instrument will indicate that the hose will be flushed. OK this command. Air will push through the hose. When everything is complete, the instrument will tell you. Do not remove the hose before being told to

do so. At this point, the melting station should be full and will fill this station. Make sure to put in new paraffin in the melting station when this station has drained it all.

1. Paraffin melting station – keep this as full as possible. Station 1-3 pull from here when they are being replaced and when the volume is low.

Sensors: Remove the metal mesh to reveal 4 sensors. Sensors need to be cleaned after every cleaning cycle. There is a cloth and a plastic stick that goes over the sensor.

Post-cleaning cycle: Remove the metal mesh to reveal 4 sensors. Sensors need to be cleaned after every cleaning cycle. There is a cloth and a plastic stick that goes over the sensor.

Placement of reagents onto drawer: reagent bottles should sit sturdily. The metal retriever goes all the way down into the bottle and the black nozzle sits over the mouth of the reagent bottle WITHOUT SEPARATION.

Drawer spills: In the event of a spill in the drawer, there is a nozzle in the bottom right corner that can be opened. The reagent will go through. Make sure to place a waste container under the nozzle.

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TACMASR OPERATING PROCEDURE

SOP #0 | Subject **Leica Autostainer XL Reagent Upkeep** | Sheet **1** of **2**

Rev 0		Effective Date	Author
10/27/2017		10/27/2017	Fimbres

1. **PURPOSE**
   The purpose of upkeeping reagents is to keep H&E stains consistent to what has been delivered previously.
1. **SCOPE**
   Cleanings/filterings/refillings should be completed weekly and when visibly necessary.
2. **REFERENCE DOCUMENTS**
3. **RESPONSIBILITIES**
   Person changing out reagents should have seen it been done before.
4. **MATERIALS and EQUIPMENT**
   Gloves
   Stainer
   Ethanol (Leica reagent grade)
   Xylene (Leica reagent grade0
   Eosin (Leica – NC9003835)
   Hematoxylin (Leica/Fisher – 3801575)
   Clearview (leica – 3803598)
   Bluing reagent leica – 3802918)

5. **SAFETY AND CAUTIONARY NOTES**
   Use gloves and fume mask when changing reagents. Dispose of waste in waste bucket/container.
6. **PROCEDURE**
   Will be outlined by station within the stainer.
   ***Keep reagents filled up to second line in each reagent station

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1	Load	Keep this station clear
2	Oven	Keep this station clear
3	Xylene	Change out every week.
4	Xylene	Change out every week.
5	Xylene	Change out every week.
6	100% EtOH	Change out every week.
7	100% EtOH	Change out every week.
8	95% EtOH	Change out twice a week or when color is changing
9	80% EtOH	Change out twice a week or when color is changing
10	Wash 1	Keep this station clear
11	Wash2	Keep this station clear
12	Hematoxylin	Filter every week.
13	Wash 3	Keep this station clear
14	Clearview	Change out every two weeks or color turns form pale yellow to sunflower yellow
15	Wash 4	Keep this station clear.
16	Bluing	Change out every two weeks or when color turns from given blue to green/blue. Filter when visibly necessary (rare)
17	Wash 5	Keep this station clear.
18	80% EtOH	Change out twice a week or when color starts to become pink. Fill up to 2nd line frequently.
19	Eosin	Filter every week.
20	100% EtOH	Remove, fill and put fresh 100% in station 15 every ~100 slides or 3 full racks. Don't allow color to reach clear eosin color.
21	100% EtOH	Move to station 13 when station 13 is replaced
22	100% EtOH	Move to station 14 when station 14 is moved
23	Xylene	Filter every week. Change every two weeks.
24	Xylene	Filter every week. Change every two weeks.
25	Xylene	Filter every week. Change every two weeks.
26	Xylene	Filter every week. Change every two weeks.

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TACMASR OPERATING PROCEDURE

SOP #0	Subject	**Microtome Cleaning and Maintenance**	Sheet	**1**	of	**1**

Rev 0		Effective Date	Author
10/27/2017		10/27/2017	Fimbres

1. PURPOSE

The purpose of this SOP is to keep the microtome and microtome area tidy. This prevents malfunctions of the microtome stemming from simple incomplete actions.

2. SCOPE

Cleaning of the microtome should happen at the end of

3. REFERENCE DOCUMENTS

4. RESPONSIBILITIES

Employees of the university with liability insurance can handle this SOP as there is risk of injury.

5. MATERIALS and EQUIPMENT

Microtome in question
Cleaning brush
ParaGuard or Xylene
70% ethanol

6. SAFETY AND CAUTIONARY NOTES

Remove blade from blade holder to prevent deep cuts.
Put brake on rotating handle.
Xylene is a carcinogen – use gloves and masks when appropriate.

7. PROCEDURE

7.1 After cutting has finished, remove tray holding paraffin scraps and dump into waste
7.2 With the cleaning brush, remove all paraffin on and around microtome.
7.3 With paraguard or xylene, remove all stubborn paraffin on and around microtome.
7.4 Remove paraguard/xylene with 70% ethanol.

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TACMASR OPERATING PROCEDURE

SOP #0 | Subject **McManus Periodic Acid Schiff Stain** | Sheet **1** of **1**

Rev 0		Effective Date	Author
10/23/2017		10/23/2017	Fimbrs

1. PURPOSE

The purpose of Periodic Acid-Schiff staining is to detect glycogen and mucosubstances in tissues to demonstrate the presence of lymphocytes and mucopolysaccharides.

2. SCOPE

Demonstrate glycogen, fungus, myucin, reticulum, basement membranes and pituitary basophil granules.

3. REFERENCE DOCUMENTS

None

4. RESPONSIBILITIES

This SOP can be carried out by someone who has seen it done before and has knowledge on the results of the tissue colors.

5. MATERIALS and EQUIPMENT

0.5% Periodic acid (dissolve 0.005g of periodic acid into 60C water and stir to dissolve)
Schiff's reagent (Newcomer supply – 1371A)
Hematoxylin (Leica/Fisher – 3801575)
Clearview (leica – 3803598)
Bluing reagent (leica – 3802918)
Oven
Running distilled water
Running tap water
70% ethanol
95% ethanol
2x 100% ethanol
3x xylene
Coverslips
Mounting media
Gloves

6. SAFETY AND CAUTIONARY NOTES

Attached

7. PROCEDURE

7.1 Bake slides for 15 minutes at 60-65 °C

7.2 Deparaffinize slides and hydrate to distilled water
7.3 Place into periodic acid for 5 minutes
7.4 Rise in distilled water 30 seconds
7.5 Place into Schiff's reagent for 15 minutes
7.6 Rinse in running tap water to develop pink color for 10 minutes
7.7 Place into hematoxylin for 30 seconds
7.8 Rinse in distilled water until water runs clear
7.9 Place into clearview for 30 seconds
7.10 Rinse in distilled water for 30 seconds
7.11 Place into bluing reagent for 15 seconds
7.12 Rinse in distilled water for 30 seconds
7.13 Dehydrate slides in increasing grades of ethanol
7.14 Clear slides with three changes of xylene
7.15 Mount and coverslip

8. Results

Rose to purple-red glycogen, mucin, fungus, basement membrane, pituitary, thyroid colloid
Blue nuclei

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PROCEDURE
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TACMASR OPERATING

Rev 0		Effective Date	Author
		5/12/2016	Kepler

1. PURPOSE

The purpose of the SOP is to establish the format for preparing tissue that will be processed on the Lieca processor.

2. SCOPE

This SOP applies to all tissue received by TAMASR that is to be processed (dehydrated, clearing and infiltrated) by the Lieca processor.

3. REFERENCE DOCUMENTS

3.1 TACMASR Request Form
3.2 "Histotechnology." Frieda L Carson. 1997
3.3 "Histotechnology." Carson & Hladik. 2009
3.4 TACMASR SOP# XXXXXXX Brady BBP11-34L
3.5 TACMASR Standard Operating Procedure #SOPXXXXX – BradyBBPII Labeler

4. RESPONSIBILITIES

Laboratory personnel preparing samples for histological processing are responsible for following the procedures in this SOP and notifying the lab director when deviations or unexpected events arise.

5. MATERIALS and EQUIPMENT

5.1 70% Ethanol	Leica 100% histology grade
5.2 10% neutral buffered formalin	Fisher 22-110-869 (Richard Allan)
5.3 Tissue Cassettes	Leica
5.4 Cassette sponges	Am Histology Reagent Co. Inc
5.5 Histology pencil	Leica
5.6 Forceps	VWR
5.7 Absorbent Pads	VWR
5.8 bio-wraps 2" x 3"	Surgipath
5.8 Brady BBPII-34L labeler	
5.8 Leica ASP6025 Advanced Processor	

6. SAFETY AND CAUTIONARY NOTES

6.1 Gloves should be worn when handling tissue and while preparing the tissue cassettes.
6.2 Spent formalin and alcohol must be discarded in the chemical waste container in the lab.

6.3 All sharps are to be discarded in the sharps container located underneath the embedder.

7. PROCEDURE

7.1 Ensure the Investigators bringing samples to the lab have included a completed TACMASR request form in total.

7.1.1 Grant number for billing, PI name, Contact name and email

7.1.2 Describe the specimen section

7.1.3 Type of project (work being requested)

7.1.4 A list of all specimen numbers

7.2 Investigator must sign in their projects in the sign in notebook located on the TACMASR intake bench. This information should include the name, date, time and number of samples.

7.3 Tacmasr staff then assign the T accession number to their TACMASR worksheet. The T accession number assign corresponds to the T number next to the investigators name on the sign in form.

7.4 Note whether samples are in 10% buffered formalin or 70% ethanol. Samples should be placed in 10% buffered formalin for up to 24 hours after they have been collected. Formalin penetrates tissue a 1mm per hour. After that time samples should be transferred to 70% ethanol to be held for processing.

7.5 TACMASR encourages investigators to fix samples in formalin for 24 hours and transfer them to 70% ethanol before bringing them to TACMASR.

7.6 For any samples brought to the lab in formalin, TACMASR staff should transfer them to 70% ethanol and note on the container 70% ethanol as well as the date and time transferred on the investigators TACMASR worksheet.

7.7 Dispose of the spent formalin in the chemical waste container located behind the fume hood.

7.8 Samples are routinely processed every Thursday afternoon.

7.9 Prepare cassette labels using the Brady Labeler and CodeSoft software associated with the computer on the desk space in 0917. Refer to TACMASR SOP# XXXXXXXX.

7.10 When the cassette label has been attached and riveted, place a blue absorbent pad on the work space, using forceps, take the sample out of the investigator vial and transfer it into the labeled cassette. If the sample is small, place it between two cassette sponges or wrap it in blue paper. Sandwiching between two sponges assures the sample remains fixed during processing and does not become lost.

7.11 Once the sample is in place, snap the cassette lid in place. The cassette lid should fit snuggly up against the cassette. It is important to make sure that no part of the sponge is preventing the lid from closing. That would cause the cassette to come apart during processing, resulting in loss of the sample.

7.12 Once the sample is in the cassette, the lid firmly attached, place the cassette into a beaker of 70% ethanol.

7.13 Repeat the process for all of the samples to be processed.

7.14 Samples are now ready to be placed on the Leica Processor, refer to SOPXXXXXXX

7.15 When finished, clean up the bench top, absorbent pad can be thrown into the trash and wash the forceps with 70% ethanol.

7.16 Spent ethanol in the investigator vials should be disposed of in the chemical waste buckets.

7.17 The investigator vials or tubes are retained for 2-3 weeks on the cart next to the marking board. In case of labeling discrepancies, the original vial can be confirmed.

7.18 The polypropylene specimen tubes/vials are discarded in the red biohazard bags, sealed and placed in the red bucket

8. RECORDS

8.1 Initial and date on the bottom of the investigator worksheet, that samples have been transferred to cassettes.

University of Arizona Cancer Center
Room 0917 Phone (520) 626-7319
UACC-TACMASS@uacc.arizona.edu

SOP #0

Subject **Picro-Sirius Red Stain**

Rev 0		Effective Date	Author
10/26/2017		10/26/2017	Fimbres

1. PURPOSE

The purpose of Picro-Sirius Red is to differentiate collagen and muscle.

2. SCOPE

3. REFERENCE DOCUMENTS

4. RESPONSIBILITIES

This SOP can be carried out by someone who has seen it done before and has knowledge of histology and knows the results of the tissue colors.

5. MATERIALS and EQUIPMENT

Weigert's Hematoxylin Parts A and B (Newcomer Supply ref#1409B)
Picro-Sirius Red stain (Fisher – NC9746363)
1% Acetic acid
Oven
Running distilled water
Running tap water
70% ethanol
95% ethanol
2x 100% ethanol
3x xylene
Coverslips
Mounting media
Gloves

6. SAFETY AND CAUTIONARY NOTES

MSDS attached

7. PROCEDURE

7.1 Bake slides for 15 minutes at 60-65 °C
7.2 Deparaffinize slides and hydrate to distilled water
7.3 Place slides into Weigert's for 5 minutes
7.4 Rinse in running tap water for 1 minute
7.5 Place slides into PSR solution for 1 hour
7.6 Rinse slides

7.7 Place slides into 1% acetic acid for 15-30 seconds (red intensity dependent)
7.8 Rinse slides in distilled water for 30 seconds
7.9 Dehydrate slides in increasing grades of ethanol
7.10 Clear slides with three changes of xylene
7.11 Mount and coverslip

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Room 0917 Phone (520) 626-7319
UACC-TACMASS@uacc.arizona.edu

TACMASR OPERATING PROCEDURE

SOP #1	Subject	**Regressive H&E staining on frozen sections**	Sheet **1** of **2**

Rev 1		Effective Date	Author
6/12/18		8/20/15	Fimbres

1. PURPOSE

The purpose of an H&E is to differentiate between purple nucleic acids (such as nucleus ofd cells) and pink proteins.

2. SCOPE

This standard operating procedure applies to all frozen sections brought to TACMASR requiring H&E staining.

3. RESPONSIBILITIES

The person carrying out this protocol should know how to operate the autostainer and have knowledge on the outcome of the tissue as per his/her pathologist prefers.

4. MATERIALS and EQUIPMENT

Leica Autostainer XL
70% ethanol
95% ethanol
2x 100% ethanol
3x xylene
Coverslips
Mounting media
Gloves
Hematoxylin (Leica/Fisher - 3801575)
Eosin (Leica – NC9003835)
Clearview (Leica – 3803598)
Bluing Reagent (leica – 3802918)

5. SAFETY AND CAUTIONARY NOTES

6. PROCEDURE

1. Frozen sections cut at 5um
2. Fixed in chilled acetone for 3 minutes
3. Hematoxylin for 2 minutes
4. Running water for 30 seconds
5. Clearview for 30 seconds
6. Running water for 30 seconds
7. Bluing for 30 seconds

8. Running water for 30 seconds
9. 70% ethanol for 1 minute
10. Eosin for 2 minutes
11. 100% ethanol for 1 minute
12. 100% ethanol for 1 minute
13. 100% ethanol for 1 minute
14. Xylene for 1 minute
15. Xylene for 1 minute
16. Xylene for 1 minute

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TACMASR OPERATING PROCEDURE

SOP #0	Subject	**Regressive Hematoxylin & Eosin for FFPE sections**	Sheet	**1**	of	**1**

Rev 2		Effective Date	Author
6/12/18		10/26/2017	Fimbres

1. PURPOSE

The purpose of an H&E is to differentiate between purple nucleic acids (such as nucleus ofd cells) and pink proteins.

2. SCOPE

This standard operating procedure applies to all FFPE sections brought to TACMASR requiring H&E staining.

3. RESPONSIBILITIES

The person carrying out this protocol should know how to operate the autostainer and have knowledge on the outcome of the tissue as per his/her pathologist prefers.

4. MATERIALS and EQUIPMENT

Leica Autostainer XL
70% ethanol
95% ethanol
2x 100% ethanol
3x xylene
Coverslips
Mounting media
Gloves
Hematoxylin (Leica/Fisher - 3801575)
Eosin (Leica – NC9003835)
Clearview (Leica – 3803598)
Bluing Reagent (leica – 3802918)

5. SAFETY AND CAUTIONARY NOTES

6. PROCEDURE

Done on the Leica AutoStainer – Program #1

1. Bake slides for 20 minutes
2. Xylene for 3 minutes
3. Xylene for 3 minutes
4. Xylene for 3 minutes
5. 100% ethanol for 2 minutes

6. 100% ethanol for 2 minutes
7. 95% ethanol for 2 minutes
8. 70% ethanol for 2 minutes
9. Running water for 30 seconds
10. Running water for 30 seconds
11. Hematoxylin for 2 minutes
12. Running water for 30 seconds
13. Clearview for 30 seconds
14. Running water for 30 seconds
15. Bluing for 30 seconds
16. Running water for 30 seconds
17. 70% ethanol for 1 minute
18. Eosin for 2 minutes
19. 100% ethanol for 1 minute
20. 100% ethanol for 1 minute
21. 100% ethanol for 1 minute
22. Xylene for 1 minute
23. Xylene for 1 minute
24. Xylene for 1 minute

Safranin O Staining Protocol for Cartilage

Description: This method is used for the detection of cartilage, mucin, and mast cell granules on formalin-fixed, paraffin-embedded tissue sections, and may be used for frozen sections as well. The cartilage and mucin will be stained orange to red, and the nuclei will be stained black. The background is stained bluish green.

Fixation: Formalin fixed, paraffin embedded sections.

Solutions and Reagents:

Weigert's Iron Hematoxylin Solution:

Stock Solution A:
Hematoxylin ---------- 1 g
95% Alcohol ---------- 100 ml

Stock Solution B:
29% Ferric chloride in water ---------- 4 ml
Distilled water ---------- 95 ml
Hydrochloric acid, concentrated ---- 1ml

Weigert's Iron Hematoxylin Working Solution:
Mix equal parts of stock solution A and B. This working solution is stable for about 4 weeks.

0.05% Fast Green (FCF) Solution:
Fast green, FCF, C.I. 42053 ---------- 0.5 g
Distilled water ---------- 1000 ml

1% Acetic Acid Solution:
Acetic acid, glacial ---------- 1 ml
Distilled water ---------- 99 ml

0.1% Safranin O Solution:
Safranin O, C.I. 50240 ---------- 0.1 g
Distilled water ---------- 100 ml

Procedure:

1. Deparaffinize and hydrate slides to distilled water.
2. Stain with Weigert's iron hematoxylin working solution for 10 minutes.
3. Wash in running tap water for 10 minutes.
4. Stain with fast green (FCF) solution for 5 minutes.
5. Rinse quickly with 1% acetic acid solution for no more than 10 –15 seconds.
6. Stain in 0.1% safranin O solution for 5 minutes.

7. Dehydrate and clear with 95% ethyl alcohol, absolute ethyl alcohol, and xylene, using 2 changes each, 2 minutes each.
8. Mount using resinous medium.

Results:

Nuclei ---------- black
Cytoplasm ---------- bluish green
Cartilage, mucin, mast cell granules ---------- orange to red

References:

1. Kahveci Z, Minbay FZ, Cavusoglu L (2000) Safranin O staining using a microwave oven. Biotech Histochem. 75(6):264-8. PubMed Abstract
2. Tran D, Golick M, Rabinovitz H, Rivlin D, Elgart G, Nordlow B (2000) Hematoxylin and safranin O staining of frozen sections. Dermatol Surg. 26(3):197-9. PubMed Abstract
3. Camplejohn KL, Allard SA. Limitations of safranin 'O' staining in proteoglycan-depleted cartilage demonstrated with monoclonal antibodies. Histochemistry. 1988;89(2):185-8. PubMed Abstract

Rev		Effective Date	Author
8/14/207 Ver5	**Policy Document for the UACC Biospecimen Repository**	2006	McDaniel/Chambers

POLICY

It is the policy of the University of Arizona Cancer Center (UACC) and the UACC Biospecimen Repository that all processes and procedures related to the acquisition, banking, and utilization of tissues and biospecimens in the Repository will comply with all applicable federal, state and University of Arizona policies and procedures.

References:
UA IRB Policy: http://rgw.arizona.edu/compliance/human-subjects-protection-program *Code of Federal Regulations (21 CFR 50) Protection of Human Subjects Office of Human Research Protection: Protection of Human Subjects (45 CFR 46).* http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.html

Project Authority: University of Arizona Cancer Center Biospecimen Repository IRB Project #06-0609-04
Setsuko K. Chambers, MD, Principal Investigator (PI)

ETHICAL AND REGULATORY COMPLIANCE REQUIREMENTS

To comply with sound research practice as required by the University of Arizona and the Food and Drug Administration regulations for the conduct and monitoring of clinical investigations, the following must be observed:

Informed Consent

The principles of informed consent and Good Clinical Practices in FDA-Regulated Clinical Trials are described in the Code of Federal Regulations (21 CFR 50) Protection of Human Subjects http://www.fda.gov/ScienceResearch/SpecialTopics/RunningClinicalTrials/default.htm, http://www.fda.gov/ScienceResearch/SpecialTopics/RunningClinicalTrials/ucm155713.htm and the Office of Human Research Protection: Protection of Human Subjects (45 CFR 46). http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.html
These regulations must be followed in conducting and monitoring clinical investigations. Consent for specimen submission will be obtained and documented in accordance with these regulations.

Institutional Review

This study must be approved by an appropriate institutional review committee as defined by Federal Regulatory Guidelines (Federal Register Vol. 46, No. 17, January 27, 1891, Part 56) and the Office of Human Research Protection. (45 CFR 46).

OVERSIGHT REQUIREMENTS

Biospecimen Oversight Committee

It is the role of the Biospecimen Oversight Committee to establish guidelines and procedures, and to provide oversight of the quality assurance management plans. The Committee ensures the overall usefulness of the Biorepository as a resource for Cancer Center researchers, and identifies where resources are insufficient to support the functions of the repository. These concerns identified by the Committee are reported to the Director of the University of Arizona Cancer Center via the UACC leadership representative. The Committee is composed of: the Principal Investigator and the Co-Principal Investigator, and Faculty representatives from the Departments of Pathology, Surgery, Surgical Oncology, and the Cancer Center. The Chair and TACMASR Biorepository Staff work together to coordinate the administrative functions of the Committee.

Quality Assurance

Quality assurance will be routinely overseen by the Oversight Committee, which meets quarterly, or more often as needed. Day-to-day activities are managed by the PI, a covering pathologist, and Biorepository staff, including the TACMASR Lab Manager, Biorepository Operations Manager and Staff. The Committee oversees the consenting and banking processes, the monitoring and quality assurance plans, tools and reports, and the acquired specimen data.

The Committee evaluates any and all proposals to utilize material from the Repository. The Committee ensures that proposals meet standards of peer reviewed research and scholarly excellence and contribute to the general body of knowledge in the fields of cancer and translational research. Final approval for requests to use any specimen comes from the Committee.

Contributing Surgeons

Contributing surgeons will have the right of first refusal for use of the specimens which they or their team have contributed from their patients. They will be contacted by the Biorepository Staff prior to consideration of release of any of their specimens. Contributing surgeons may choose whether and how they will be involved in all stages of a research project which utilizes specimens they have contributed. They are to be acknowledged as a contributing author on publications arising from the research.

Utilization of the Biorepository

The Arizona Cancer Center Biospecimen Repository is property of the University of Arizona Cancer Center. The use of tissues and biospecimens from the Repository will be available to UACC members and collaborating investigators only for specific proposals. Investigators who are interested in utilizing the Repository for specific proposals must document compliance with University of Arizona Institutional Review Board (IRB) policies and approval requirements. Investigators will then submit the proposal to the Biospecimen Oversight Committee stating the objectives of their study, preliminary data supporting their hypothesis, their research plan, and expected outcome. The proposal should represent either peer-reviewed research studies or clinical trials, or research stemming from previously peer-reviewed proposals.

Preparation of Biorepository material is handled as a routine request for service through the Tissue Acquisition and Cellular/Molecular Analysis Shared Resource (TACMASR), and is charged accordingly for work performed.

Overview of Study Processes

SOP 400-2

1/18/2019
Version 006

Rev		Effective Date	Author
8/14/2017 Ver5	**SOP for the UACC Biospecimen Repository**	2006	McDaniel

1. PURPOSE & SCOPE

To outline the overall study processes and requirements related to the activities, management, and administration of the University of Arizona Cancer Center Biospecimen Repository (Biorepository, TACMASR Tumor Bank, Tissue Bank).

2. DEFINITIONS

- Institutional Review Board (IRB): Applicable guidelines, certifications, and Verification of Training (VoTF) requirements are administered by the University of Arizona (UA) Human Subjects Protection Program, Office for the Responsible Conduct of Research, http://rgw.arizona.edu/compliance/human-subjects-protection-program
- Tissue Acquisition and Cellular/Molecular Analysis Shared Resource (TACMASR) is the UACC "core" lab that is responsible for management of the UACC Biorepository. TACMASR also provides histological and pathological services to UACC investigators. http://uacc.arizona.edu/research/shared-resources/tacmasr
- The University of Arizona Health Network UAHN, (formerly University Medical Center, UMC)
- Banner University Medical Center – Tucson (BUMCT)
- TissueMetrix (AIM) is the biospecimen information management system administered through the University of Arizona Cancer Center.

3. REFERENCES

Project Authority: University of Arizona Cancer Center Biospecimen Repository IRB Project #06-0609-04
Setsuko K. Chambers, MD, Principal Investigator

Documents, SOPs, and associated references related to the Biorepository

- *IRB F200 Application for Human Research* (F200 Project Approval September 29, 2011, updated 2015)
- 400-1 Policies Governing the UACC Biospecimen Repository
- SOP 400-2 Overview of Study Processes (including a procedural flowchart)
- SOP 400-3 Oversight Committee: Role and Responsibilities
- SOP 400-4 Biorepository Personnel: Roles and Responsibilities
- SOP 400-5 Steps to Enroll Subjects and Bank Biospecimens
- SOP 400-6 Monitoring Plan and Tools for Quality Assurance (including education & training)
    - Tissue Bank Instructions for Clinic Staff
    - Monitor/Audit Plan
- SOP 400-7 Utilization of Biospecimens
- SOP 400-8 Consenting Process
- SOP 400-9 Information Management
    - TissueMetrix Screenshots: Overview and Workflow Labeling System
    - AZR Collection Event Log Form
- SOP 400-10 Tissue Acquisition and Processing
    - AZR Frozen Sample Box Log Forms for biospecimens
- SOP 400-11 Preparation of Blood Samples
    - AZR Frozen Sample Box Logs –Whole blood, Plasma, Serum

4. PROCEDURAL FLOWCHART: STEPS AND RESPONSIBLE PERSONNEL FOR THE BANKING PROCESS.

Step	Personnel
1. Identify potential subject	Surgeon or TACMASR staff
↓	
2. Obtain informed consent	Surgeon, Surgeon's staff or TACMASR staff
↓	
3. Notify TACMASR of consent & DOS	Surgeon's staff
↓	
4. Transport consent documents to TACMASR	Surgeon or Research staff
↓	
5. Verify consent documents	Biospecimen Operations Manager
↓	
6. Assign Subject PT ID	Biospecimen Operations Manager
↓	
7. Assign Collection Number to specimens	Biospecimen Operations Manager
↓	
8. Collect surgical tissues and body fluids	Biospecimen Operations Manager
↓	
9. Label, process and archive tissues/fluids	Biospecimen Operations Manager
↓	
10. Enter biospecimen data on log forms	Biospecimen Operations Manager
↓	
11. Confirm histological quality of tissues	Pathologist
↓	
12. Enter data into TissueMetrix	TACMASR Staff
↓	
13. File documents	TACMASR Staff
↓	
14. Distribute biospecimens	TACMASR Staff

5. OVERVIEW OF STUDY PROCESSES AND RELATED STANDARD OPERATING PROTOCOLS (SOPs)

5.1 Oversight of the UACC Biospecimen Repository

The Biospecimen Oversight Committee establishes policies, procedures, and guidelines for the Study, and oversees all aspects of quality assurance, processes, management, and utilization of specimens.

400-1 Policies Governing the UACC Biospecimen Repository
SOP 400-3 Biospecimen Oversight Committee: Roles and Responsibilities

5.2 Overview of Qualifications and Personnel

Detailed descriptions of the roles and responsibilities that are required for Study personnel are included in SOP400-4. Qualifications, effort, duties, reporting structures, and the monitoring plan and tools for each position are outlined in an Excel document. Personnel who are currently filling all positions are identified in this SOP.

All Study personnel must have current CITI certification and must be listed on the current VOTF. Surgeons and their designees who consent patients also must have CITI certification and be listed on the current VOTF.

SOP 400-4 Personnel: Roles and Responsibilities

5.3 Overview of Monitoring Plans and Tools

The original management plan for the Biorepository that was in effect from 2006 to 2011 was significantly reorganized in the summer/fall of 2011. The revised policies and management and regulatory adherence plans that are now in effect were approved by the Biospecimen Oversight Committee and the IRB in the fall of 2011.

NOTE: Biorepository SOPs that were in effect from 2006-2011 are labeled Version 1. As part of the 2011 reorganization of the Biorepository, the Version 1 SOPs (in effect from 2006-2011) were thoroughly updated and are labeled Version 2. New SOPs that were written as part of the 2011 reorganization are labeled Version 1. In 2014, the SOPs were again thoroughly edited and updated, and are labeled with the subsequent version number (2 or 3).

SOPs are reviewed and updated annually or as needed. All versions are retained as part of the Biospecimen Repository. Current versions are available on the TACMASR lab shared drive and are printed in a Current SOPs Binder notebook that is easily accessible to Study Staff.

SOP 400-6 Monitoring Plan and Tools for Quality Assurance

5.4 Overview of Subject Privacy, Security and Confidentiality

The policies and procedures incorporated in this Study emphasize privacy, confidentiality and security at every step. Study personnel must be qualified and adequately trained. Information is managed in a secure database, and data and documents are stored in a secure location.

SOP 400-6 Monitoring Plans and Tools
SOP 400-9 Information Management

5.5 Overview of the Consenting Process

A procedural flowchart is included at the beginning of this SOP400-2. Briefly, Surgeons identify potential Subjects and discuss the Study with them prior to surgery. The surgeon, or their delegated personnel, obtain informed consent and notify Tumor Bank personnel. The Biorepository Coordinator and Operations Manager verify that consent documents are properly signed and dated. SOP400-8 describes the consenting process in detail. SOP400-5 describes the step-by-step chronological plan for the consenting and enrolling process, and the monitoring plan and tools for each step.

SOP 400-8: Consenting Process
SOP 400-5: Steps to Enroll Subjects and Bank Biospecimens

5.6 Overview of the Specimen Collection Process

The Biorepository Operations Manager and assistants collect specimens from Banner University Medical Center Pre-op clinics and surgical suites during normal business hours: 8AM-5PM Monday through Friday. Special arrangements must be made with the Biorepository Operations Manager to collect and process specimens after hours. Biospecimens are processed immediately after pickup for long term storage.

The Pathology Department's Attending Pathologist, Resident or Physician Assistant identifies small portions of tissues they deem not to be necessary for diagnosis, but which are otherwise adequate for tissue banking. The Biospecimen Operations Manager prioritizes and fixes the tissue specimens, depending on quantity of tissue available, as follows:

1) Snap freeze OCT Frozen
2) Formalin fix, paraffin embed (FFPE).

Hematoxylin and Eosin (H&E) slides are prepared for each tissue specimen and reviewed by a certified Pathologist. Preservation quality and tissue histology are documented.

Two 6ml Vacutainer tubes of whole blood are drawn by the Pre-op staff: one plasma tube (EDTA anticoagulant) and one serum tube (no anticoagulant), thus allowing for whole blood, EDTA plasma and serum to be banked.

NOTE: In June, 2013, the Oversight Committee approved the suspension of collection and preservation of urine aliquots. They have received no requests for the use of urine specimens since inception. The Committee reserves the right to reinstate collection at any time.

NOTE: In June, 2011 smaller Vacutainer tubes of blood started being drawn, resulting in the preservation of two vials of serum or plasma containing 1ml each.

NOTE: The collection and preservation of tissue specimens in RNALater was also dropped, ~ 2013.

NOTE: Urine collections were started again August 2018 for Dr. Chipollini's cases and then October 2018 for Dr. Lee's cases.

SOP 400-10 Tissue Acquisition and Processing
SOP 400-11 Preparation of Blood Samples

5.7 Overview of the Management Information System

TissueMetrix is the biospecimen management software that is used by the University of Arizona Cancer Center to track biospecimens, from acquisition to long term storage. TissueMetrix is a critical tool in UACC's efforts to effectively manage, track, and document information to ensure patient privacy.

Several individual biorepositories (silos) are consolidated into this "umbrella" data management system. The program is encoded, requires a login and password, and allows for restricted access to identifiable information (such as subject name, MRN, DOB, etc.). The system allows for restricted access to aggregate information. Individual silos are password protected and managed independently by personnel authorized to enter and view private information solely from their silo. The Database Administrator sets up individual accounts, trains authorized users, and provides ongoing database maintenance and troubleshooting. Information is searchable and reports can be created.

The "Storefront" application within TissueMetrix displays a real time inventory of the number and types of tissues/tumors that are available for use in translational research via the University of Arizona Cancer Center's website. UACC Researchers are able to access general information about banked specimens through the TissueMetrix Storefront.

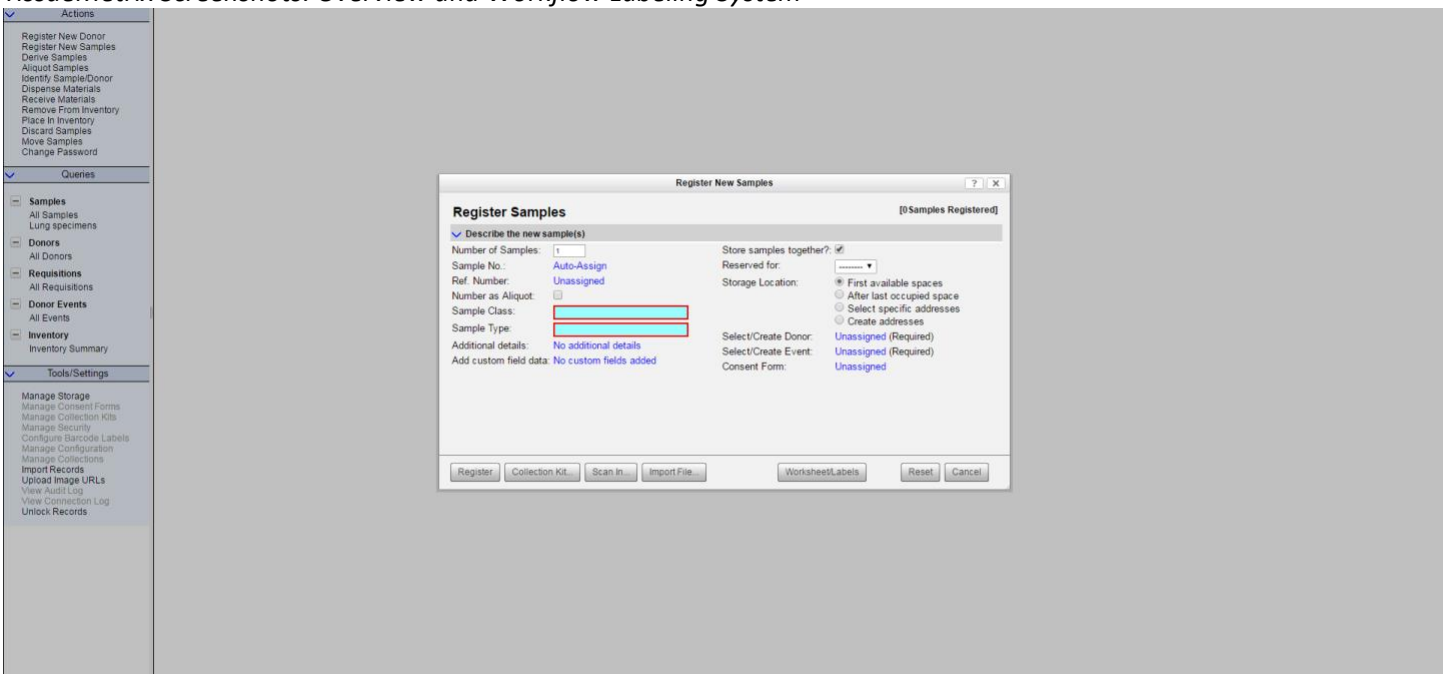
With the implementation of TissueMetrix on January 1, 2011, enrolled Subjects are assigned accession codes generated by TissueMetrix. Subjects who were enrolled in the Biorepository from inception in 2006 through the end of 2010 have been reassigned an accession code that is compatible with TissueMetrix but retains the original accessioning and specimen labeling system that was in use at the time.

SOP 400-9 Information Management

5.8 Overview of the Biorepository Labeling and Barcoding System

Subjects are assigned a randomly generated identification number. Biospecimens are assigned sequential de-identified numbers which are barcoded and tracked in TissueMetrix. Refer to a visual flowchart in the SOP below, for a screenshot depiction of the TissueMetrix management system, and log forms and barcoded labeling system.

SOP 400-9 Information Management
TissueMetrix Screenshots: Overview and Workflow Labeling System



5.9 Overview of the Biospecimen Utilization Process

UACC members and collaborating investigators who are interested in utilizing specimens from the Biorepository may submit a specific proposal to the Biospecimen Oversight Committee, stating the specific objectives of the study, preliminary data supporting the hypothesis, the research plan, and expected outcomes. IRB compliance is required.

The application form is routed through TACMASR, which prepares specimens that meet the investigator's criteria on a fee-for-service basis.

SOP 400-7: Utilization of Biospecimens

Rev		Effective Date	Author
11/6/19 Ver7	**SOP for UACC Biospecimen Repository**	2007	McDaniel

1. PURPOSE & SCOPE

To define the membership, current members and reporting structure of the Oversight Committee; to describe the roles and responsibilities of the Committee; to outline the reporting structure of the Biorepository management and quality assurance program; and to outline utilization of biospecimens and materials.

2. DEFINITIONS

- Institutional Review Board (IRB): Applicable guidelines, certifications, and Verification of Training (VoTF) requirements are administered by the University of Arizona (UA) Human Subjects Protection Program, Office for the Responsible Conduct of Research, http://rgw.arizona.edu/compliance/human-subjects-protection-program
- TACMASR: Tissue Acquisition and Cellular/Molecular Analysis Shared Resource http://uacc.arizona.edu/research/shared-resources/tacmasr

3. REFERENCES

Project Authority: University of Arizona Cancer Center Biospecimen Repository IRB Project #06-0609-04
Setsuko K. Chambers, MD, Principal Investigator

- 400-1 Policies Governing the UACC Biospecimen Repository
- SOP 400-2 Overview of Study Processes
- SOP 400-4 Biorepository Personnel: Role and Responsibilities
- SOP 400-5 Steps to Enroll Subjects and Bank Biospecimens
- SOP 400-6 Monitoring Plan and Tools for Quality Assurance
- SOP 400-7 Biospecimen Utilization and Application Process
- SOP 400-8 Consenting Process
- SOP 400-9 Information Management

4. MEMBERSHIP AND CURRENT SITTING MEMBERS

The Committee is composed of: the Principal Investigator and Faculty representatives from the Departments of Pathology, Surgery, Surgical Oncology, Urology, UACC and TACMASR.

Voting Members of the Biospecimen Oversight Committee

Current Chair and Principal Investigator	Setsuko K. Chambers, MD UACC Physician-Scientist, Director of Women's Cancers Professor and Vice-Chair of Obstetrics and Gynecology
Chair, Department of Pathology	Achyut Bhattacharyya, MD, Pathologist Professor of Pathology

Division Chief, Anatomic Pathology	Erika Bracamonte, MD Pathologist Associate Professor, Pathology
Faculty representative, Surgery	Ron Heimark, PhD, UACC Researcher Professor and Vice Chair for Research, Department of Surgery
Division Chief, Urology	Benjamin Lee, MD Professor, Department of Surgery
Consulting Study Pathologist	Belinda Sun, MD, PhD Clinical Assistant Professor, Pathology
Faculty representative, Surgical Oncology	Rebecca Viscusi, MD Assistant Professor, Department of Surgical Oncology
Faculty representative, Surgical Oncology	James Warneke, MD, FACS Associate Professor, Department of Surgery

Non-voting, ad hoc members of the Oversight Committee

UACC Leadership	Ioannis Stasinopoulos Research Development Administrator	Remove
Shared Resources Director	Ronald Lynch, PhD	
TACMASR Director	Yana Zarvos	
Biorepository Coordinator/Operations Manager	Carole Kepler, BS, MT(ASCP) Research Specialist, Principal TACMASR Staff	
Admin/Regulatory Coordinator	Mitzi Miranda, BS, Division of Women's Cancers	

5. REPORTING RELATIONSHIP TO THE UNIVERSITY OF ARIZONA CANCER CENTER

The Biospecimen Oversight Committee reports to the Director of the University of Arizona Cancer Center.

6. ROLE AND RESPONSIBILITIES OF COMMITTEE

Role

The role of the Committee is to establish policies, procedures and guidelines for the UACC Biospecimen Repository that meet federal and regulatory requirements. The Committee ensures the overall usefulness of the Biorepository as a resource for Cancer Center researchers, and identifies appropriate resources to support the functions of the repository. The concerns identified by the Committee are reported to the Director of the Arizona Cancer Center via the UACC leadership representative.

Responsibilities

Quality control and assurance will be overseen by the UACC Biospecimen Repository Oversight Committee. The day-to-day management and oversight will be managed by the Principal Investigator,, TACMASR Lab Manager, Tumor Bank Coordinator, Operations Manager and staff. The Committee meets quarterly or more often as needed. The committee may also conduct business by email, as appropriate.

The Committee's quality assurance program includes a yearly, independent review of the Biorepository conducted by a knowledgeable and objective employee of the Cancer Center who is appointed by the Committee. The appointment of the Biospecimen Repository Quality Assurance Reviewer is approved by the Director of the University of Arizona Cancer Center.

The Committee evaluates any and all proposals to utilize material from the Repository. The Committee ensures that proposals submitted by UACC investigators and their collaborators meet standards of scientific merit and scholarly excellence, and contribute to the general body of knowledge in the fields of cancer and translational research.

The Committee is responsible for oversight of the following

- The consenting and banking processes
- The monitoring and quality assurance plans, tools, and reports, and implementing any corrective actions to the quality assurance management plan
- The acquired specimen data
- The utilization of specimens and materials

The functions of this committee include

- Addressing issues related to the management and functioning of the bank
- Identifying and communicating with the UACC Leadership and Director that funding and personnel resources are adequate and provided
- Reviewing and responding to patient, surgeon, or medical staff complaints
- Determining whether IRB requirements have been met
- Evaluating number of patients enrolled
- Evaluating the quality of tissues and biospecimens that have been banked
- Appointing an UACC Quality Assurance Reviewer and evaluating the report prepared by the Reviewer
- Evaluating and approving/disapproving requests for use of specimens

7. QUALITY ASSURANCE (WHO/WHAT REPORTS TO THE COMMITTEE)

The Chair and the TACMASR Director and Biorepository Staff work together to coordinate and carry out biospecimen management and administrative functions of the Biorepository.

Members of the Biorepository and Regulatory Staff are responsible for

- Performing administrative duties requested by the Committee including preparation of documents, notification of meetings, agenda, reports and minutes of scheduled meetings.
- Reporting administrative and management issues to the Committee, recommending potential solutions to solve issues, and implementing Committee instructions
- Preparing summary reports of subjects enrolled and whether all IRB requirements have been met.
- Reporting any consenting and procedural issues to the Committee, recommending potential solutions to solve issues, and implementing Committee instructions.
- Facilitating and processing applications for use of banked biospecimen material from UACC investigators and ensuring that investigators' requests comply with policies and procedures adopted by the Biospecimen Oversight Committee.

The UACC Biorepository Quality Assurance Reviewer reports to the Committee

- Reviewer conducts a yearly, independent review of the Biorepository for quality assurance using a monitoring program developed specifically for the Biorepository/Tumor bank.
- The review will be conducted annually, generally in January prior to submission of the Annual Review to the IRB.
- The Committee reviews and evaluates results, conclusions and suggestions from the Reviewer's report.
- The outcome of the review is incorporated into the Annual Continuing Review, which is generally due to the IRB in February.
- *Refer to SOP 400-6 "Audit Program"- Monitor / Review of AZCC Tumor Bank,* for details of audit process and procedures.

8. UTILIZATION OF BIOSPECIMENS

The Arizona Cancer Center Biospecimen Repository is property of the University of Arizona Cancer Center. The use of tissues and biospecimens from the Repository will be available to UACC members and collaborating investigators only for specific proposals.

The Committee evaluation process requires that

- If applicable, investigators who are interested in utilizing the Repository for specific proposals must document compliance with University of Arizona Institutional Review Board (IRB) policies and approval requirements
- Investigators then submit an application form to the Biospecimen Oversight Committee, stating the objectives of their study, preliminary data supporting their hypothesis, a research plan and the expected outcome. Proposals should represent either peer-reviewed research studies or clinical trials, or research stemming from previously peer-reviewed proposals.
- The Committee reviews and evaluates the scientific merit, objectives, preliminary data supporting the hypothesis, research plan, and expected outcome of the proposed study.
- Final approval/disapproval for requests to use any specimen comes from the Committee, and requires a majority vote of the membership.
- The approval process may be conducted by email in order to expedite investigators' requests for use of biospecimens.

Refer to SOP 400-7: Utilization of Biospecimens

Rev		Effective Date	Author
01/03/20 Ver5	**SOP for UACC Biospecimen Repository**	2007	McDaniel

1. PURPOSE & SCOPE

To define the consenting procedures for the UACC Biospecimen Repository.

2. DEFINITIONS

- Institutional Review Board (IRB): Applicable guidelines, certifications, and Verification of Training (VoTF) requirements are administered by the University of Arizona (UA) Human Subjects Protection Program, Office for the Responsible Conduct of Research, http://rgw.arizona.edu/compliance/human-subjects-protection-program

- TACMASR: Tissue Acquisition and Cellular/Molecular Analysis Shared Resource http://uacc.arizona.edu/research/shared-resources/tacmasr

3. REFERENCES

Project Authority: University of Arizona Cancer Center Biospecimen Repository
IRB Project #06-0609-04, Setsuko K. Chambers, MD, PI

SOP 400-2 Overview of Study Processes
SOP 400-5 Steps to Enroll Subjects and Bank Biospecimens (Steps 1 – 5)
SOP 400-6 Monitoring Plan and Tools for Quality Assurance
SOP 400-9 Information Management
APPENDIX 1 Key points to address when consenting participants.
IRB approved consent documents with current dated stamp

4. RESPONSIBILITIES

4.1 Study personnel who have access to Subject samples and information as part of their job duties are responsible for following the procedures in the SOP in order to protect subject privacy and confidentiality at all times.

4.2 The PI, TACMASR Director, ~~TACMASR Lab Manager~~, Biorepository Operations Manager/Coordinator, and Study personnel are responsible for education and training of surgeons, their designated personnel who identify, recruit and obtain informed consent.

4.3 The PI, TACMASR Director, Biorepository Operations Manager , and Lab Manager are responsible for education and training of laboratory research personnel who are involved with the study as part of their job duties.

4.4 Deviations are to be reported immediately to the PI and ~~Co-PI~~ TACMASR Director.

5. PROCEDURAL FLOWCHART OF THE CONSENTING PROCESS

Step	Personnel
1. Identify potential subject	Surgeon/TACMASR staff
↓	
2. Obtain informed consent	Surgeon/TACMASR staff
↓	
3. Provide a copy of original signed consent to participant	Surgeon's staff
↓	
4. Notify TACMASR of consent & DOS	Surgeon's staff
↓	
5. Transport consent documents to TACMASR Biorepository staff	Surgeon or Surgeon's staff
↓	
6. Verify consent documents	Biorepository Coordinator

6. CONSENTING PROCEDURES

STEP 1: PROCEDURES TO IDENTIFY POTENTIAL SUBJECTS

1. Potential subjects are identified by their surgeon/physician prior to surgery
2. Potential subjects are recruited at the time decisions and plans regarding their surgery or biopsy are being discussed
3. The Surgeon discusses the purposes and procedures with the potential Subject

STEP 2: PROCEDURES TO OBTAIN INFORMED CONSENT

1. The Surgeon, or their designee, and TACMASR staff obtains the informed consent authorization of the Subject. It is the responsibility of the presenter to thoroughly explain what is being asked of the patient and to present them with the current informed consent document to read. Refer to Appendix 1 of this SOP for key points to address.
2. Potential subjects are given time to read the consent forms and discuss the Study with their surgeon or Presenter
3. The Subject is asked to sign the consent, and to indicate their response to each of the five questions on Page 2
4. The Surgeon signs the consent authorization documents as the Investigator
5. A designee who presents the documents may sign as the Presenter
6. To ensure that the correct dated and stamped consent forms are being used, the ~~Tu~~ TACMASR Internal regulatory coordinator distributes the current documents to each surgeon's group (nurse managers, administrative staff), on brightly colored paper (Light Blue for Mar 6, 2019-Mar 6, 2022)
7. The preferred protocol is to print consent documents on colored paper; however, TACMASR accepts properly signed and dated documents that are printed on white paper
8. A copy of the signed consent form is to be given to the participant

STEP 3: PROCEDURES TO NOTIFY TACMASR THAT INFORMED CONSENT HAS BEEN OBTAINED

1. Each surgeon or their nurse manager or administrative staff notifies TACMASR that informed consent has been obtained from a Subject and the tentative Date of Surgery (DOS).
2. They are requested to notify TACMASR, prior to surgery, by email at UACC-TACAMSR@uacc.arizona.edu

STEP 4: PROCEDURES TO TRANSPORT INFORMED CONSENT DOCUMENTS TO TACMASR

1. Consent documents are transported from the Surgeons' offices to TACMASR in sealed envelopes prior to the date of surgery.
2. Consent documents may be mailed to TACMASR through interdepartmental mail, addressed to the TACMASR, UACC PO Box 245024, Room 0914.
3. Consent documents may be transported by TACMASR Research Staff personnel or surgeon's personnel.
4. Coordinator may also pick up consents in designated locations.

STEP 5: PROCEDURES TO VERIFY CONSENT DOCUMENTS

1. Prior to the date of surgery, the Tumor Bank Coordinator confirms the receipt of original, and ensures the consent is properly signed by the subject and surgeon.
2. The Coordinator contacts the surgeon/designee if documents are not complete and maintains communication until issues are resolved for a period of six months. The Coordinator holds on to the consent documents until issues are resolved.
3. The Coordinator submits the documents to the Manager of TACMASR weekly for verification.
4. If there is no resolution within six months, the PI and Co-PI will be informed and will contact the surgeon prior to discarding the specimens.

APPENDIX 1

-Banner University of Medical Center is a research and teaching hospital affiliated with the University of Arizona Cancer Center.

-The University of Arizona Cancer Center is an NCI (National Cancer Institute) designated Comprehensive Cancer Center, one of 41 in the country.

-A requirement of being an NCI designated Comprehensive Cancer Center is to have a biorepository, in other words a big tissue bank.

-Because the surgeons at Banner University Medical Center participant in the tissue banking process, we are asking that a small piece of tissue that is removed during surgery be banked for future research. No additional tissue will be removed from you other than what is being removed as your standard of care. The tissue that pathology (the department of physicians that diagnosis your condition) doesn't need would be deemed extra tissue and is generally discarded. We ask if a small piece of that tissue could be put into the tissue bank.

-Tissue that goes into this bank can be used by the researchers at the University of Arizona Cancer Center.

-This tissue is de-identified; a number is put on it so that any researcher using your tissue will not know your name.

-Study personnel will look at your medical records to get your age, ethnicity and the pathology results (what your final diagnosis is).

-We will also take a small syringe of blood, this is done in pre-op when your IV is started, thus no additional needle stick will be required.

-You do not benefit from being in the study

-There is NO cost to you for being in the study

-Frequently, the participants will question to agreeing to follow up check mark box. Stress they have the right to refuse this if they choose or to be removed from participating at any time.

-After signing, offer to make them a copy of the signed consent.

Current personnel & qualification	Role and % effort to Biorepsoitory	Tumor Bank responsibilities	Work location	Reports to	Monitoring plan and tools for quality assurance
PI: Setsuko K. Chambers MD UACC Physician Scientist and Attending Surgeon	Principal Investigator, Chair of the Biospecimen Oversight Cmte	Directs Biospecimen Oversight Committee, which establishes policies and mission/vision, and approves use of repository material. Provides oversight of Biospecimen Repository. Oversees IRB compliance and clinical consenting processes, and liaisons with surgeons and their designees. Works with TACMASR and the Biorepsitory staff to address and resolve issues.	UACC Room 4921 Office	UACC Director	***Plan:*** Ensure that the Biorepository adheres to policies and mission. ***Tools:*** PI is identified on IRB Form 200 and the VoTF. Refer to SOP 400-3: Biospecimen Oversight Committee
Belinda Sun MD Covering Attending Pathologist		Provides QA/QC monitoring of the quality of preserved specimens.	UACC	UACC Director	***Plan:*** Ensure quality assurance/quality control of specimens. ***Tools :*** The quality of banked tissues is monitored by review of H&E stains of each tissue by a qualified Pathologist.
Oversight Committee Voting Members: Setsuko Chambers, MD; Amanda Baker, PhD, PharmD; Achyut Bhattacharyya, MD; Erika Bracamonte, MD; Ron Heimark, PhD; James Warneke, MD; Rebecca Viscusi, MD; Benjamin Lee, MD	**Members represent stakeholders from UACC and UAHN.** Committee oversees all aspects of the Biorepository	Establishes policies and ensures that resources are adequate to fulfill the mission and maintain compliance with IRB and regulatory entities. Evaluates and approves/disapproves applications for use of banked biospecimens. Addresses consenting, banking, and quality assurance status and issues related to the management and functioning of the bank.	N/A	UACC Director and Senior Leadership	***Plan:*** Establishes policies, procedures and guidelines for the UACC Biospecimen Repository that meet federal and regulatory requirements and oversees and enforces compliance. ***Tools:*** Meets quarterly. Conducts relevant business by email if applicable.

Current personnel & qualification	Role and % effort to Biorepsoitory	Tumor Bank responsibilities	Work location	Reports to	Monitoring plan and tools for quality assurance
Carole Kepler, BS, MT (ASCP) **Biorepository Coordinator/ Operations Manager** Research Specialist, Principal	Biospecimen Operations Manager 1.0 FTE	Oversee and manage all aspects of the biospecimen acquisition, processing, preservation,, distribution and quality control. This includes but is not limited to monitoring surgical oncology OR schedules for resectable cancer cases, preparing the weekly collection schedule, consenting patients, collection and procssing of blood specimens from pre-op and the OR, coordinating with pre-op and OR staff for specimen pickup, blood processing and storage, tissue pickup in the OR and transportation of it to pathology for grossing, tissue preservation & storage, ensuring the collection event log is completed in entirety, assigns and manages storage locations of all biospecimens, maintains specimen box logs, maintains all -80 freezers, daily coordination with clinical pathology physicians, residents and assistants for tissue grossing, conducts ongoing education and training with support personnel including clinical staff, maintains updated SOP's, and provides a quarterly progress report for the oversight committee. Performs daily & ongoing internal monitoring of consent documents & records. Monitors daily data management processes for privacy compliance, accuracy & quality control. Reports consenting, procedural and management issues to the Director, PI, and oversight committee. Oversees the input and verifies patient identifiers and collection, tracking and annotation data via TissueMetrix. Maintains lab copies, electronic and paper, of all documents related to the Biorepository. Coordinates with UACC Internal Monitor to provide requested documents, informationand interview as part of the periodic Monitoring/Review program. Facilitates applications for use of banked materials by UACC investigators. This process includes: working with investigators to select and prepare biospecimens that meet investigator's criteria and needs,ensuring that investigators' requests comply with policies and procedures adopted by the Biospecimen Oversight	UACC TACMAS Labs Rooms UACC TACMASR Labs Rooms 0915 & 0917, Office 0914, and BUMC Pre-Op & pathology	Director of TACMASR, PI of the Biorepository, TACMASR , Biospecimen Oversight Committee	***Plan:*** Ensure that Coordinator is qualified and understands the policies and procedures related to this tissue banking study. Ensure that Coordinator receives training to perform job duties, and obtains all necessary certifications and appropriate level of security and training to perform job duties, including access to patient information. Coordinator adheres to all SOPs. ***Tools:*** Coordinator is listed on the VoTF, and has received Login/password to UAMC Patient Information Management system (EPIC), and applications that are relevant to the Biorepository. Coordinator is trained and has login/password access to TissueMetrix management information system. Refer to SOPs.
Jocelyn Fimbres, BS Research Specialist	Histologist, Procurment 0.2 FTE	Prepares FFPE blocks and Hematoxylin & Eosisn (H & E) slides for review by TACMASR Pathologist. Maintains histology equipment & performs late afternoon tissue collections, assists the Biospecimen Operations Manager when needed.	UACC TACMASR Labs 0915,0917 Office 0914 BUMC pre-op, frozen section room and BUMC Morgue	Director of TACMASR, Lab Manager, Operations Manager	***Plan:*** Ensure that equipment and resources that support banking efforts are properly maintained. Ensure that Study personnel are properly trained for job duties. Study personnel adhere to all SOPs. ***Tools:*** Study personnel are listed on the VoTF

Current personnel & qualification	Role and % effort to Biorepsoitory	Tumor Bank responsibilities	Work location	Reports to	Monitoring plan and tools for quality assurance
Mitzi Miranda, BS Program Coordinator, Senior **Division of Women's Cancers**	Division of Women's Cancers	Assists Dr. Chambers as needed for work related to the Biorepository. Works with the Biospecimen Oversight Committee to coordinate administrative duties including preparation of documents, coordinating updates to SOPs, notification of meetings, agenda and minutes of scheduled meetings,takes notes, and prepares the Minutes for Biospecimen Oversight Committee meetings. Works with the Biorepository Staff to provide assistance related to the Biorepository as needed. Conducts all correspondence with the IRB, including Annual Reviews, VOTFs, and Approval forms. Reports consenting, procedural and management issues to the PI and the IRB. Coordinates with the (Biorepository Tumor Bank) Coordinator to obtain and maintain current information for all IRB documentation. Maintains master copies of all IRB documentation for the Biorepository. Attends Biospecimen Oversight Committee meetings.	Dr. Chambers' office UACC 4921	Dr. Chambers	**Plan:** Ensure that Program Coordinator is qualified and trained, and understands the policies and procedures related to this tissue banking study.
Manuel Snyder, MSc Senior Programmer TissueMetrix Administrator	Database administrator and manager of TissueMetrix information system	Establishes login and password protected accounts. Provides ongoing database maintenance and troubleshooting.	UACC Office Room 1928	Director of Bioinformatics Shared Service, PI and Co-PI of the UACC Repository	**Plan:** Ensure that Study data is managed using a web based, verifiable management system (TissueMetrix. **Tools:** The TissueMetrix program is encoded, requires a login and password, and allows for restricted access to identifiable information (such as subject name, MRN, DOB, etc.).
Mary Krutszch Research Specialist Sr **Independent Quality Assurance Monitor/Reviewer**	Biorepository Quality Assurance Monitor/Reviewer This Position is appointed by Oversight Cmte	Conducts an annual review of the Biorepository (generally in January) in order to ascertain whether the required policies and procedures governing the collection of human subject biospecimens by the UACC Biospecimen Repository are being followed as defined by the SOPs and approved by the Biospecimen Oversight Committee and the UA IRB, consistent with the stated goals, objectives, and current regulations.	UACC	Biospecimen Oversight Committee	**Plan:** Ensure that the Biospecimen Oversight Committee has appointed a knowledgeable and objective employee of the Cancer Center to conduct the review. Ensure that periodic, internal monitoring of the Biorepository are conducted. **Tools**: See SOP "Audit Program"- Monitor / Review of UACC Biorepository, for details of audit process and procedures.

SOP 400-5: Steps to Enroll Subjects and Bank Biospecimens

Step	Process	Person(s) responsible	Procedure	Location
1	Identify potential subjects	Surgeon	Potential subjects are identified by their surgeon prior to surgery. Any surgical patient over the age of 18 and able to give informed consent is eligible for the study.	Clinics (Banner-University Medical Center and North Campus), Pre-op clinics
2	Obtain Informed consent	Surgeon or their designee (research nurse or research coordinator), and/or Biorepository personnel who are qualified to obtain consent	Surgeon or their designee, or qualified Biorepository personnel, discusses the study (or studies) with patient and obtains informed consent for each study. To ensure that the correct, dated, stamped Consent forms are being used by each group, the Tumor Bank Coordinator personally distributes documents to surgeons/nurse managers/administrative personnel, with verbal and/or written instructions to shred outdated documents and begin using renewed ones on the correct dates. Coordinator prints consent documents on colored paper and changes the color annually (the color light blue from Xerox was chosen for the April 2019-April 2020 consenting period). The preferred protocol is to print the consent documents on the colored paper; however, the Biorepository accepts properly signed and dated documents that are printed on white paper.	Clinics (Banner-University Medical Center and North Campus), Pre-op clinics
3	Notify the Biorepository of consent and DOS	Surgery personnel	Each surgeon/designee notifies the Biorepository of (tentative or confirmed) DOS for consented patients.	Via email or phone contact
4	Transport Consent documents	Surgeon's designated personnel	Consent documents are transported in sealed manila envelopes addressed to the TACMASR, UACC 0914. Biorepository personnel may also pick up consent documents from a designated place in each surgical group's office. Alternatively, documents are delivered by surgeon's personnel or mailed or transported by courier from Banner-University of Arizona North Campus.	From clinics or pre-op to TACMASR (UACC 0914) via personal delivery, interdepartmental mail, or courier.

SOP 400-5: Steps to Enroll Subjects and Bank Biospecimens

Step	Process	Person(s) responsible	Procedure	Location
5	Verify consent documents	Biorepository (Tumor Bank) Coordinator (Carole Kepler)	Coordinator confirms the receipt of original, stamped, properly signed Subject's Consent. Coordinator contacts appropriate surgeon/designee if documents are not complete. Maintains communication until issues are resolved. Coordinator brings unresolved issues, after six months, to PI, Regulatory Coordinator, Lab Manager, or to the Oversight Committee for further action, and follows up with indicated resolution. Coordinator submits documents to the Lab Manager (or Dr. Chambers) weekly for verification. Coordinator notifies PI of violations and coordinates with the Regulatory Coordinator to prepare a report.	UACC TACMASR 0914
6	Assign Subject ID and derived Specimen ID	Biorepostory personnel	Biorepository personnel assigns a unique barcoded Subject ID Number (aka PTID Number), which is randomly generated. The PTID number is linked to a Collection Event Number, and each Collection Event Number (R16XXXXX) is linked to a series of coded Specimen ID numbers/barcodes which track each individual biospecimen (R16XXXXXX).	UACC TACMASR 0914
7	Prepare to collect surgical specimens	Biospecimen Operations Manager or Assistant	Biospecimen manager notifies Pre-op of consented patients. Pre-op staff collect two tubes of blood, one red top and one lavendar top, when starting the patient's IV. Pre-op pages the Biorepository for blood pickup.	UACC TACMASR Lab 0914. Frozen section room is located in Banner-University Medical Center adjacent to the OR suites. Morgue and Pre-op are located in Banner-University Medical Center.

Step	Process	Person(s) responsible	Procedure	Location
8	Collect surgical specimens and body fluids	Biospecimen Operations Manager or Assistant	The Pathology Attending, Resident or PA identifies small portions of tissues they deem not to be necessary for diagnosis, but otherwise adequate for tissue banking. Biorepository Operations Manager/Assistant prioritizes and fixes the specimens, depending on quantity of tissue available: 1) snap freeze 2) Formalin fix, paraffin embed (FFPE).	Frozen section room or Banner-University Medical Center morgue
9	Label and process tissues and fluids	Biospecimen Operations Manager or Assistant	For each Collection Event and each individual specimen, Biorepository personnel obtain pre-printed PTID, collection event and specimen ID barcode labels. Lab personnel process and label biospecimens for long term preservation (serum, plasma, whole blood are aliquotted, and tumor/tissues are snap frozen or made into FFPE blocks). Collection data, subject information, and dispensation data are recorded on the Subject's Collection Event log form and Sample type log forms. Duplicate labels are affixed to Sample-type log forms to assign and record the storage location (eg, freezer/rack/box). Laboratory personnel places specimens into appropriate storage locations.	UACC TACMASR Lab 0915
10	Enter biospecimen data on Log forms	Biospecimen Operations Manager or Assistant	Biorepository personnel document collection processes, dispensation data and storage locations on the Study log forms (Collection Event and Sample type).	TACMASR labs UACC 0915, and -80C freezer rooms in the basement of Salmon building

Step	Process	Person(s) responsible	Procedure	Location
11	Confirm histological quality of tissue	TACMASR Histologist, Pathologist	TACMASR Histologist cuts and stains an H&E slides on each frozen tissue specimen and each formalin fixed, paraffin embedded (FFPE) tissue that is banked. Pathologist evaluates each slide to assess 1) morphology 2) presence of normal or cancer 3) type and grade of cancer, and 4) preservation quality. Coordinator enters H&E slide results in TissueMetrix.	TACMASR lab and office 0915 and 0914, and -80C freezer rooms
12	Enter data into TissueMetrix	Tumor Bank Coordinator, or Assistant	Biorepository staff enters information gathered from Log Forms, Pathology reports, and from tissue slides that are prepared by the Repository histology personnel (H&E slides made from each frozen and paraffin tissue block entered into the bank)	UACC TACMASR 0914
13	File documents	Tumor Bank Coordinator	TB Coordinator files study documents in 3-ring binders; each subject's file includes properly signed consent forms, Collection Event and Sample Type log forms, and pathology report.	UACC TACMASR room 0914 / Medical Records Room 0903
14	Distribute biospecimens	Tumor Bank Coordinator	Coordinator searches TissueMetrix to identify repository material to meet investigator's criteria. Specimens are prepared and routed through the TACMASR core service.	UACC TACMASR 0914

Monitoring plan and tools for quality assurance
Plan: Ensure that surgeon receives adequate training with regard to policies and procedures of the Biorepository. **Tools:** Verify that Surgeon is listed on VoTF. Educate surgeons/designees and OR personnel via personal conversation, in-services, written instructions and website. Distribute Handout to Clinic Staff (flyer). Refer to *SOP: 400-2 Overview of Study Processes.*
Plan: Ensure that surgeon/designees and Biorepository personnel receive adequate training with regard to policies and procedures of the Biorepository. **Tools:** Verify that Surgeon/designees/Biorepository personnel who consent patients are listed on the VoTF. . Distribute Handout to Surgeons (flyer). Verify that Subject signed properly using the current, dated and stamped consent forms.
Biorepository personnel verifies prior notification information against actual consent documents that are received. *Refer to SOP 400-8 Consenting Process.*
Plan: Ensure that transport systems protect the privacy of patient information. **Tools:** Transport documents in sealed envelopes. Ensure that each surgeon's personnel have the correct address/email/contact information for TACMASR. TACMASR maintains and updates a Telephone/email Contact List for internal use.

Monitoring plan and tools for quality assurance
Plan: Ensure that informed consent documents are properly signed and dated, and verified by Bioreposiotry Coordinator. **Tools:** TACMASR Lab Manager also verifies consent documents weekly. Coordinator prepares summary report to present to Oversight Committee. PI reports problems and corrective actions to IRB (F224) and follows up with indicated resolution.
Plan: Ensure that information is managed and coded in a secure electronic database to protect Subject privacy and confidentiality. Ensure that access to information is restricted. **Tools:** Use the TissueMetrix information management system to track information and biospecimens from Subjects entered into the Biorepository. Refer to *SOP 400-9 Information Management* and the Labeling TissueMetrix Workflow.
Plan: Ensure that OR Personnel have been educated to page/call the Bioreposiotry only if the subject has been consented for this study. **Tools:** Biorepository personnel again verify that they have received properly signed documents in order to confirm the OR's call for pickup of surgical specimens/fluids. Refer to *SOP 400-2 Overview of Study Processes* for the tools used by the Research staff to educate and train clinical staff.

Monitoring plan and tools for quality assurance
Plan: Obtain and preserve tissues and body fluids as rapidly as possible to ensure that optimal biospecimens are banked in the Repository. **Tools:** Transport supplies to the Frozen Section room in order to preserve "on site" and as rapidly as possible, generally within 30 minutes. Refer to *SOP 400-10 Tissue Processing, and SOP 400-11 Preparation of Blood Samples.*
Plan: Ensure that sufficient resources are available to preserve high quality specimens for future translational research. Ensure that specimens are de-identified, and that collection details are documented for future research. **Tools:** Refer to SOP 400-10 to harvest and prepare tissues/tumors and SOP 400-11 to harvest body fluids for banking. Barcoded labels de-identify each sample that is banked. *Refer to* SOP 400-9 Information Management, for TissueMetrix labeling system information.
Plan: Biorepository personnel verifies that each individual sample is labeled, de-identified, and stored in the proper location, and that all information on log forms is complete and accurate. **Tools:** Input data from log forms into the TissueMetrix information management system. Refer to *SOP 400-9 Information Management, SOP 400-10 Tissue Processing* and *SOP 400-11 Preparation of Blood Samples*

Monitoring plan and tools for quality assurance
Plan: Verify the quality and pathological diagnosis of each tissue specimen that is banked. **Tools:** TACMASR Pathologist assesses the histology and preservation quality on each tissue by reviewing the H&E slide.
Plan: Ensure that Biorepository staff have obtained proper training and has received access to Banner-University Medical Center patient information management system and to the TissueMetrix data management system. **Tools:** *Refer to SOP 400-9 Information Management* and *SOP 400-10 Tissue Processing*
Plan: Verify that authorized personnel have key access to TACMASR labs. **Tools:** Refer to SOP: Monitoring Plan and Tools for Quality Assurance - Security
Plan: Verify that an investigator who is interested in utilizing specimens has obtained IRB approval and Oversight Committee approval. **Tools:** Refer to *SOP 400-7 Utilization of Biospecimens,* which describes the approval and application processes required by the Oversight Committee in order to distribute de-identified biospecimens to researchers.

SOP # **400-5**

Steps to Enroll Subjects and Bank Biospecimens

Rev		Effective Date	Author
8/14/17 Ver5	**SOP for the UACC Biospecimen Repository**	2006	McDaniel

1. PURPOSE & SCOPE

To provide a chronological workflow plan and step-by-step requirements, from enrolling and consenting subjects into the Repository, to collection, preservation, banking and documenting of biospecimens.

2. DEFINITIONS

- Institutional Review Board (IRB): Applicable guidelines and certifications, and Verification of Training (VoTF) requirements are administered by the University of Arizona (UA) Human Subjects Protection Program, Office for the Responsible Conduct of Research, http://orcr.arizona.edu
- Tissue Acquisition and Cellular/Molecular Analysis Shared Resource (TACMASR) is the "core" lab that is responsible for management of the UACC Biospecimen Repository (Tumor Bank). TACMASR also provides histological and pathological services to UACC investigators. http://azcc.arizona.edu/research/shared-services/tacmass
- Banner- University Medical Center medical records are accessed through the Citrix management information system.
- TissueMetrix (AIM) is the biospecimen information management administered through the University of Arizona Cancer Center.

3. REFERENCES

Project Authority: Arizona Cancer Center Biospecimen Repository IRB Project #06-0609-04
Setsuko K. Chambers, MD, Principal Investigator

- SOP 400-2: Overview of Study Processes
- SOP 400-4: Biorepository Personnel: Role and Responsibilities
- SOP 400-6: Monitoring Plan and Tools for Quality Assurance

4. RESPONSIBILITIES

It is the responsibility of laboratory personnel preparing samples for banking to follow the procedures in the SOP. Deviations are to be documented on the Collection Event Form. Unexpected events are to be reported to the PI, Director and Lab Manager.

An Excel spreadsheet with detailed steps is part of this document.

Rev		Effective Date	Author
1/19/19 Ver7	**SOP for the UACC Biospecimen Repository**	2011	McDaniel

1. PURPOSE & SCOPE

To outline a quality assurance management plan, and the monitoring plans and tools, to ensure that the required policies and procedures governing the University of Arizona Cancer Center Biospecimen Repository (Tumor Bank) are being followed and documented.

2. DEFINITIONS

- Institutional Review Board (IRB): Applicable guidelines and certifications, and Verification of Training (VoTF) requirements are administered by the University of Arizona (UA) Human Subjects Protection Program, Office for the Responsible Conduct of Research, http://rgw.arizona.edu/compliance/human-subjects-protection-program
- Tissue Acquisition and Cellular/Molecular Analysis Shared Resource (TACMASR) is the "core" lab that is responsible for management of the UACC Biospecimen Repository (Tumor Bank). TACMASR also provides histological and pathological services to UACC investigators. http://uacc.arizona.edu/research/shared-resources/tacmasr
- The Banner- University Medical Center medical records are accessed through the CITRIX management information system.
- TissueMetrix (AIM) is the biospecimen information management administered through the University of Arizona Cancer Center.

3. REFERENCES

Project Authority: University of Arizona Cancer Center Biospecimen Repository IRB Project #06-0609-04
Setsuko Chambers, MD, Principal Investigator

- 400-1 Policies Governing the UACC Biospecimen Repository
- SOP 400-2: Overview of Study Processes
- SOP 400-3: Oversight Committee: Roles and Responsibilities
- SOP 400-4: Biorepository Personnel: Role and Responsibilities
- SOP 400-5: Steps to Enroll Subjects and Bank Biospecimens
- SOP 400-7 Utilization of Biospecimens

4. PROCESSES, AND MONITORING PLANS AND TOOLS FOR QUALITY ASSURANCE

4.1 Qualification of Study Personnel

Each surgeon who contributes to the Biorepository is responsible for assigning and managing his/her own personnel.

Monitoring plan

- Ensure that TACMASR Study personnel are qualified to perform assigned roles and responsibilities.

Monitoring tools

- Refer to SOP 400-4: *Biorepository Personnel: Roles and Responsibilities* which defines the qualifications required for TACMASR research laboratory personnel.

4.2 Education and Training of Study personnel

Education and training of Study personnel is an ongoing effort and often requires individualized formats and forums designed to meet specific aspects of the Study.

Monitoring plan

- Ensure that TACMASR research personnel are qualified to carry out their roles and responsibilities related to the Biorepository.
- Ensure that educational opportunities and training sessions are conducted with all personnel who are involved with this Study to ensure that each person understands and adheres to policies and procedures governing the Study.

Monitoring tools

- Refer to *SOP 400-5: Steps to Enroll Subjects and Bank Biospecimens* which describes the education and training used at each step of the banking process.
- All Study personnel are required to pass the CITI online course, which provides foundational information related to human subjects research and privacy protection.
- All study personnel are required to take Basic Biosafety Training with the ORCBS/Blood Borne Pathogens
- The Biospecimen Operations Manager/Tumor Bank Coordinator conducts ongoing education and training sessions with contributing surgeons and their designated staff (clinic staff, nurse managers, admins), and with research laboratory personnel.
- Ongoing training sessions may include one-on-one discussions, presentations at Operating Room staff In-services, handouts, and SOPs.

4.3 Privacy and Security

Maintaining the privacy, confidentiality and security of Subject information is of utmost importance in this Study.

Monitoring plan

- Ensure that Subject privacy is protected at each step during the consenting, collection, banking and utilization processes.

Monitoring tools

- Refer to *SOP 400-2 Overview of Study Processes for the UACC Biospecimen Repository*
- Refer to *SOP 400-5: Steps to Consent and Bank Biospecimens*
- The importance of maintaining patient privacy and protection is stressed throughout all education and training materials and sessions with surgeon/designees and Research staff.
- Study personnel who require access to protected patient information in order to fulfill their duties must obtain the required training from the appropriate UAHN patient information management system administrator(s) in order to be granted login and password access to the system.
- Study personnel who require access to the TissueMetrix data management system in order to log and track biospecimens must obtain privileges from the TissueMetrix Administrator in order to obtain the appropriate level of login and password access.

- Lab Manager verifies that only authorized personnel have key access to TACMASR rooms where patient information and documents are managed, logged, and filed.
- Consenting and privacy issues are reported to the Director/PI and Lab Manager, and may also be referred to the Biospecimen Oversight Committee. Study personnel may recommend potential solutions to solve issues, and implement Director's and Committee instructions.
- Problems and corrective actions are reported to the IRB (*Form 224 Reportable Local Information Items That Are Potentially Problematic).*

4.4 SOPs

It is the responsibility of TACMASR laboratory personnel who are collecting and preparing samples to follow the procedures in the SOPs.

Monitoring plan

- Ensure that SOP 400-5, which documents the steps from identification of potential subject to long term banking, is being executed properly
- Ensure that tissues and body fluids are collected and banked following protocols *SOP 400-10 Tissue Acquisition and Processing,* and *SOP 400-11 Preparation of Blood Samples*
- Ensure that corrective actions are taken
- Ensure that all SOPs are reviewed and revised annually

Monitoring tools

- Follow the specific Monitoring Plan and Tools that are to be used in each step of the SOPs
- Document deviations and unexpected events on the Collection Event
- Report deviations and unexpected events to the Coordinator, Lab Manager, PI and Oversight Committee
- Report deviations and unexpected events to the IRB on the appropriate form
- Implement corrective actions
- All versions of SOPs are retained electronically and in a master file in TACMASR. Current versions are readily accessible to Study Staff and monitors.

4.5 Biospecimen Storage

The actual biospecimens and associated information that are stored and banked to form the Biorepository are invaluable. Frozen biospecimens are stored in UltraLow freezers at -80C, and must never be allowed to thaw.

Monitoring plan

- Ensure that all types of materials that comprise the Biorepository are materials are stored properly and securely
- Ensure that UltraLow freezers are continuously monitored with an electronic alarm system
- Verify that UACC and BUMC Facilities maintains adequate resources to ensure that UltraLow freezer alarm systems are reliable and effective
- Ensure that alarm systems on the UltraLow freezers are connected to the Banner University Medical Center Security Office and/or to an authorized monitoring contractor

Monitoring tools

- The UltraLow freezer alarms systems monitor the temperature on a continuous basis, iMonnett

- A temperature that is out of range initiates an alarm in the BUMC Security office & a text message to the Biospecimen Operations manager.
- The Lab Manager ensures that Security has a current "Call List" of TACMASR laboratory personnel who are to be contacted if an alarm is set off
- Security is instructed to call and speak to someone on the list, starting at the top of the Call List
- Security is instructed to continue calling down the list, leaving messages, until they speak to a staff person
- Upon receipt of a call from Security, personnel must respond immediately to assess the situation and solve the issue
- The Call List is also posted on the door of each freezer
- The Biorepository consists of 14 UltraLow freezers (TACMASR specimens, Cancer Prevention & Control and Nagle research specimens are also stored in these -80C freezers)
- Paraffin blocks and H&E slides are filed/stored in the TACMASR labs in the Cancer Center (Room 0915)
- Original consent documents and Event Log forms in notebook binders are securely stored in rooms with limited and monitored access.

4.6 Biospecimen Retrieval

Applications for biospecimens, once approved, will be retrieved by TACMASR staff, verified for accuracy and provided to the requestors.

Monitoring plan

- To ensure that the specimens provided to users match the criteria they provided in their application
- To maintain accurate data records in TissueMetrix on incoming and outgoing specimens

Monitoring tools

- Training of appropriate staff and students will be provided by the Biospecimen Operations Manager
- Database search for available specimens that meet the request's criteria will be performed
- Specimens for each request will be pulled by one member of the TACMASR staff
- For Quality Assurance, another member of the TACMASR staff will compare the specimens to the Collection Event Log to confirm accuracy
- Once the order has been confirmed as complete and accurate, the requestor will be provided with the specimens
- SOP *400-7 Utilization of Biospecimens* provides detail on the application process for requesting specimens
- SOP *400-9 Information Management* provides detail on the data management software, TissueMetrix, as well as information to be collected and entered into the system

4.7 UACC Monitor/Audit Plan for Quality Assurance

In addition to daily monitoring and internal auditing by the Tumor Bank Coordinator and Lab Manager, the University of Arizona Cancer Center Director and Executive committee implemented a Monitor/Review Plan in August, 2011.

Monitoring plan

- Conduct an annual review of the Biospecimen Repository, by an independent UACC reviewer.
- Ensure that the required policies and procedures governing the collection of human subject biospecimens by the UACC Biospecimen Repository are being followed as defined and approved by the UA IRB, consistent with the stated goals, objectives, and current regulations.

- Ensure that the Biospecimen Oversight Committee has appointed a knowledgeable and objective employee of the University of Arizona Cancer Center to conduct the annual review, generally in January.
- Ensure that the UACC Reviewer submits a report of findings to the Biospecimen Oversight Committee for approval and corrective action.

Monitoring tools

- Follow all procedures described in SOP 400-6: Monitor/review Audit Steps
    1) Review documents
    2) Conduct interviews
    3) Select a random sample of 10% Subjects who have been enrolled in the Study to review and document discrepancies
    4) Review minutes of Biospecimen Oversight Committee since last meeting
    5) Prepare a report summarizing the findings of the audit, including check list, and submit to Oversight Committee.

4.8 IRB Project Approval and Continuing Review

The original project approval for *the University* of *Arizona Cancer Center Biospecimen Repository* was approved by the IRB, using the format that was in use at the time, entitled *Project approval form for ethical review of activities involving human biological/genetic materials.* The original Project Start date was September 1, 2006.

An updated Project Approval F200 was approved by the IRB in October 2011, using the current form in use: *IRB Form 200: Application for Human Research*.

Monitoring plan

- Ensure that human subject research involving the UACC Biospecimen Repository is carried out under the approval of the University of Arizona Institutional Review Board's (IRB) authority. http://rgw.arizona.edu/compliance/human-subjects-protection-program
- Ensure that the annual IRB Continuing Review for this Study is approved.

Monitoring tools

- *IRB Form Renewal/Closure for Human Subjects* and associated documents are due to the IRB office 45 days prior to the expiration date.
- The Regulatory Coordinator works with the P.I. and Tumor Bank Coordinator regulatory personnel to prepare and submit the Continuing Review report, and to follow up as needed. The Tumor Bank Coordinator tabulates the number of patient samples acquired and supplies any other information needed to complete the annual progress report.
- The Regulatory Coordinator also submits any other correspondence to the IRB, including maintaining an updated VoTF form and any information that is reportable to the IRB.
- Signatures are obtained from authorized signers.
- Re-approved, dated Annual Review, VoTF and consent forms are filed in Dr. Chambers' office and TACMASR (electronically and in a master file).

4.9 IRB Certifications and Verification

The University of Arizona conducts the majority of its research compliance training, including human subject privacy and protection, through the Collaborative Institutional Training Initiative (CITI), an online modular based training program.

http://rgw.arizona.edu/compliance/human-subjects-protection-program . Certifications for human subject research are active for four years.

Monitoring plan

- Ensure that all surgeons and their designees who consent patient for this Study have active CITI certification. Surgeons/designees/admins are responsible for maintaining current certification.
- Ensure that TACMASR Director and Personnel who are involved with this Study and have access to patient identifiers have active CITI certification.
- All Study personnel must be included on the current VoTF.
- The Tumor Bank Coordinator and Lab Manager verify that all Study personnel are listed on the VoTF, and that the VoTF is updated whenever new Study personnel are added or removed.

Monitoring tools

- Obtain IRB approval for all personnel changes to the VoTF: Submit request for changes as they occur to the IRB using *Form F213 Modification of Approved Human Research.*
- Include any changes on a master VoTF: *Form F107 Verification of Human Subjects Training Form (VoTF).*
- The Clinical Trials office of the UACC maintains a database of active CITI dates of certification for all research personnel.

5.0 BUMC Site Review Authority

Banner University Medical Center reviews and approves/disapproves all research conducted in facilities that are within its purview.

Monitoring plan

- Ensure that the BUMC Site Review Authority (SRA), which grants UAHN approval for the continuance of this Study, is active and current.

Monitoring tools

- SRA approval is obtained as required, and is documented in the annual Continuing Review.

5.1 Statements of Confidentiality

The Banner University Medical Center Network reviews and approves/disapproves all research conducted in facilities that are within its purview.

Monitoring plan

- Ensure that a current Statement of Confidentiality is on file for each member, including Directors, of the TACMASR lab.

Monitoring tools

- Copies of the Confidentiality agreements for each person are on file in the TACMASR office, in the "Employee Competency" notebook.

5.2 Utilization of Banked Material

The overall goal of this Study is to provide cancer investigators access to a wide variety of optimally preserved tissues and biospecimens, along with essential pathologic information needed for translational studies in cancer.

Monitoring plan

- Ensure that UACC investigators and their collaborators have access to Study material

Monitoring tools

- Refer to the UACC Biospecimen Repository Policy document 400-1
- The *SOP 400-7 Utilization of Biospecimens* describes the criteria, requirements and processes that investigators are required to follow in order to utilize biospecimens and associated information from the Biorepository.
- The Biospecimen Oversight Committee evaluates and approves/disapproves requests from investigators to utilize banked materials
- TACMASR maintains a master file (electronic and paper) of applications that were approved by the Oversight Committee for use of repository material, and a master log of the applications.

Rev		Effective Date	Author
1/15/19 Ver6	**SOP for UACC Biospecimen Repository**	2007	McDaniel/Nagle/ Chambers

1. PURPOSE & SCOPE

To outline the application process required to request the use of biospecimens, as defined by the Oversight Committee.

2. DEFINITIONS

- Institutional Review Board (IRB): Applicable guidelines, certifications, and Verification of Training (VoTF) requirements are administered by the University of Arizona (UA) Human Subjects Protection Program, Office for the Responsible Research Conduct of Research, http://rgw.arizona.edu/compliance/human-subjects-protection-program
- TACMASR: Tissue Acquisition and Cellular/Molecular Analysis Shared Resource is the "core" lab that is responsible for management of the UACC Biospecimen Repository (Tumor Bank). TACMASR also provides histological and pathological services to UACC investigators. http://uacc.arizona.edu/research/shared-resources/tacmasr

3. REFERENCES

Project Authority: Arizona Cancer Center Biospecimen Repository IRB Project #06-0609-04
Setsuko K. Chambers, MD, Principal Investigator

- 400-1 Policies Governing the UACC Biospecimen Repository
- SOP 400-3: Biospecimen Oversight Committee: Roles and Responsibilities
- Application Form Request for Use of Biospecimens

4. APPLICATION PROCESS FOR USE OF BIOSPECIMENS

The Arizona Cancer Center Biospecimen Repository is the property of the University of Arizona Cancer Center and will be available to UACC members and collaborating investigators for specifically identified and approved proposals ONLY.

4.1 Proposals to utilize banked materials should represent either peer-reviewed research studies or clinical trials, or research stemming from previously peer-reviewed proposals.

4.2 It is the responsibility of the investigator to determine if the proposed project is considered human research, and thus requires IRB approval, prior to applying to the Repository (Form Determination of Human Research).

4.3 A copy of any IRB correspondence approving the research project must be submitted to the Biorepository, along with the completed Application form *Request for Use of Biospecimens.*

4.4 Refer to the IRB website for instructions, forms and a link to the investigators manual: http://rgw.arizona.edu/compliance/human-subjects-protection-program

4.5 Note that that the IRB does not require submission of Determination of Human Research form, for the use of de-identified biospecimens.

4.6 However, a completed, Determination of Human Research form must be included in the application packet in order to obtain de-identified materials from the Biorepository.

4.7 To request an Application Form, contact TACMASR by email at UACC-TACMASS@uacc.arizona.edu

The form is also available on the TACMASR website http://uacc.arizona.edu/research/shared-resources/tacmasr

4.8 The investigator applicant includes a detailed description of the criteria and the amount of material that is requested.

4.9 The Tumor Bank Coordinator will assist the investigator with the application process and with preparation of biospecimens that meet their research objectives. The Tumor Bank Coordinator will confirm that banked material which meets the investigator's criteria is available and sufficient to meet the request.

4.10 Biorepository personnel will request that the investigator contact the contributing surgeon to discuss the proposed studies utilizing material they have contributed from their surgical patients.

4. 11 The Tumor Bank Coordinator will work with the investigator to ensure that the investigator's criteria for tissue (e.g.; type, grade, quality) are met.

4.12 The Tumor Bank Coordinator submits the completed application form to the Oversight Committee via email.

4.13 The Biospecimen Oversight Committee evaluates the objectives of the proposed study, preliminary data supporting the hypothesis, the research plan and the expected outcomes, and approves/disapproves the application by a majority vote.

4.14 The Tumor Bank Coordinator will follow up with the investigator regarding the Committee's response.

4.15 Preparation of material is handled as a routine request for service through the Shared Resource, TACMASR, and is charged accordingly for work performed.

Refer to 400-1 Policies Governing the UACC Biospecimen Repository: Contributing Surgeons

Contributing surgeons will have the right of first refusal for use of the specimens which they or their team have contributed from their patients. They will be contacted by the Biorepository Coordinator prior to consideration of release of any of their specimens. Contributing surgeons may choose whether and how they will be involved in all stages of a research project which utilizes specimens they have contributed. They are to be acknowledged as a contributing author on publications arising from the research.

5. BIOSPECIMEN OVERSIGHT COMMITTEE: CURRENT SITTING MEMBERS IN 2017

Principal Investigator: Setsuko Chambers, MD, PI and Chair, UACC Physician-scientist, Director of Women's Cancers, Professor and Vice-Chair of Obstetrics and Gynecology

Department of Pathology Chair: Achyut Bhattacharyya, MD, Pathologist, Professor of Pathology
Division Chief, Anatomic Pathology: Erika Bracamonte, MD, Pathologist**,** Associate Director of Pathology
Consulting Pathologist: Belinda Sun, MD, PhD, Clinical Assistant Professor
Faculty representative, Surgery: Ron Heimark, PhD, UACC Cancer Biology Researcher, Professor and Vice Chair for Research, Department of Surgery
Faculty representative, Urology: Benjamin Lee, MD, Professor, Surgery
Faculty representative, Surgical Oncology: Rebecca Viscusi, MD, Assistant Professor, Surgery
Faculty representative, Surgical Oncology: James Warneke, MD, Associate Professor, Surgery, Vice chair Education/Professionalism
Faculty representative, TACMASR Director: Yana Zavros PhD, Professor, Cellular & Molecular Medicine, Associate Head for Research

~~**Ad hoc, UACC Leadership, Ioannis Stasinopoulos, PhD, Research** Development Administrator~~
Ad hoc TACMASR acting Director, Ronald Lynch, PhD Associate Director Shared Resources
Ad hoc, Tumor Bank Coordinator/Operations Manager, Carole Kepler, BS, MT(ASCP), Research Specialist, Principal
Ad hoc, Regulatory Coordinator, Mitzi Miranda, BS, Senior Program Coordinator

Rev		Effective Date	Author
01/03/20 Ver5	**SOP for UACC Biospecimen Repository**	2007	McDaniel

1. PURPOSE & SCOPE

To define the consenting procedures for the UACC Biospecimen Repository.

2. DEFINITIONS

- Institutional Review Board (IRB): Applicable guidelines, certifications, and Verification of Training (VoTF) requirements are administered by the University of Arizona (UA) Human Subjects Protection Program, Office for the Responsible Conduct of Research, http://rgw.arizona.edu/compliance/human-subjects-protection-program

- TACMASR: Tissue Acquisition and Cellular/Molecular Analysis Shared Resource http://uacc.arizona.edu/research/shared-resources/tacmasr

3. REFERENCES

Project Authority: University of Arizona Cancer Center Biospecimen Repository
IRB Project #06-0609-04, Setsuko K. Chambers, MD, PI

SOP 400-2 Overview of Study Processes
SOP 400-5 Steps to Enroll Subjects and Bank Biospecimens (Steps 1 – 5)
SOP 400-6 Monitoring Plan and Tools for Quality Assurance
SOP 400-9 Information Management
APPENDIX 1 Key points to address when consenting participants.
IRB approved consent documents with current dated stamp

4. RESPONSIBILITIES

4.1 Study personnel who have access to Subject samples and information as part of their job duties are responsible for following the procedures in the SOP in order to protect subject privacy and confidentiality at all times.

4.2 The PI, TACMASR Director, ~~TACMASR Lab Manager~~, Biorepository Operations Manager/Coordinator, and Study personnel are responsible for education and training of surgeons, their designated personnel who identify, recruit and obtain informed consent.

4.3 The PI, TACMASR Director, Biorepository Operations Manager , and Lab Manager are responsible for education and training of laboratory research personnel who are involved with the study as part of their job duties.

4.4 Deviations are to be reported immediately to the PI and ~~Co-PI~~ TACMASR Director.

5. PROCEDURAL FLOWCHART OF THE CONSENTING PROCESS

Step	Personnel
1. Identify potential subject	Surgeon/TACMASR staff
↓	
2. Obtain informed consent	Surgeon/TACMASR staff
↓	
3. Provide a copy of original signed consent to participant	Surgeon's staff
↓	
4. Notify TACMASR of consent & DOS	Surgeon's staff
↓	
5. Transport consent documents to TACMASR Biorepository staff	Surgeon or Surgeon's staff
↓	
6. Verify consent documents	Biorepository Coordinator

6. CONSENTING PROCEDURES

STEP 1: PROCEDURES TO IDENTIFY POTENTIAL SUBJECTS

1. Potential subjects are identified by their surgeon/physician prior to surgery
2. Potential subjects are recruited at the time decisions and plans regarding their surgery or biopsy are being discussed
3. The Surgeon discusses the purposes and procedures with the potential Subject

STEP 2: PROCEDURES TO OBTAIN INFORMED CONSENT

1. The Surgeon, or their designee, and TACMASR staff obtains the informed consent authorization of the Subject. It is the responsibility of the presenter to thoroughly explain what is being asked of the patient and to present them with the current informed consent document to read. Refer to Appendix 1 of this SOP for key points to address.
2. Potential subjects are given time to read the consent forms and discuss the Study with their surgeon or Presenter
3. The Subject is asked to sign the consent, and to indicate their response to each of the five questions on Page 2
4. The Surgeon signs the consent authorization documents as the Investigator
5. A designee who presents the documents may sign as the Presenter
6. To ensure that the correct dated and stamped consent forms are being used, the ~~Tu~~ TACMASR Internal regulatory coordinator distributes the current documents to each surgeon's group (nurse managers, administrative staff), on brightly colored paper (Light Blue for Mar 6, 2019-Mar 6, 2022)
7. The preferred protocol is to print consent documents on colored paper; however, TACMASR accepts properly signed and dated documents that are printed on white paper
8. A copy of the signed consent form is to be given to the participant

STEP 3: PROCEDURES TO NOTIFY TACMASR THAT INFORMED CONSENT HAS BEEN OBTAINED

1. Each surgeon or their nurse manager or administrative staff notifies TACMASR that informed consent has been obtained from a Subject and the tentative Date of Surgery (DOS).
2. They are requested to notify TACMASR, prior to surgery, by email at UACC-TACAMSR@uacc.arizona.edu

STEP 4: PROCEDURES TO TRANSPORT INFORMED CONSENT DOCUMENTS TO TACMASR

1. Consent documents are transported from the Surgeons' offices to TACMASR in sealed envelopes prior to the date of surgery.
2. Consent documents may be mailed to TACMASR through interdepartmental mail, addressed to the TACMASR, UACC PO Box 245024, Room 0914.
3. Consent documents may be transported by TACMASR Research Staff personnel or surgeon's personnel.
4. Coordinator may also pick up consents in designated locations.

STEP 5: PROCEDURES TO VERIFY CONSENT DOCUMENTS

1. Prior to the date of surgery, the Tumor Bank Coordinator confirms the receipt of original, and ensures the consent is properly signed by the subject and surgeon.
2. The Coordinator contacts the surgeon/designee if documents are not complete and maintains communication until issues are resolved for a period of six months. The Coordinator holds on to the consent documents until issues are resolved.
3. The Coordinator submits the documents to the Manager of TACMASR weekly for verification.
4. If there is no resolution within six months, the PI and Co-PI will be informed and will contact the surgeon prior to discarding the specimens.

APPENDIX 1

-Banner University of Medical Center is a research and teaching hospital affiliated with the University of Arizona Cancer Center.

-The University of Arizona Cancer Center is an NCI (National Cancer Institute) designated Comprehensive Cancer Center, one of 41 in the country.

-A requirement of being an NCI designated Comprehensive Cancer Center is to have a biorepository, in other words a big tissue bank.

-Because the surgeons at Banner University Medical Center participant in the tissue banking process, we are asking that a small piece of tissue that is removed during surgery be banked for future research. No additional tissue will be removed from you other than what is being removed as your standard of care. The tissue that pathology (the department of physicians that diagnosis your condition) doesn't need would be deemed extra tissue and is generally discarded. We ask if a small piece of that tissue could be put into the tissue bank.

-Tissue that goes into this bank can be used by the researchers at the University of Arizona Cancer Center.

-This tissue is de-identified; a number is put on it so that any researcher using your tissue will not know your name.

-Study personnel will look at your medical records to get your age, ethnicity and the pathology results (what your final diagnosis is).

-We will also take a small syringe of blood, this is done in pre-op when your IV is started, thus no additional needle stick will be required.

-You do not benefit from being in the study

-There is NO cost to you for being in the study

-Frequently, the participants will question to agreeing to follow up check mark box. Stress they have the right to refuse this if they choose or to be removed from participating at any time.

-After signing, offer to make them a copy of the signed consent.

Rev		Effective Date	Author
8/16/17 Ver5	**SOP for UACC Biospecimen Repository**	2014	Kepler

APPENDIX 1

Key Points to address when consenting a potential participant:

-Banner University of Medical Center is a research and teaching hospital affiliated with the University of Arizona Cancer Center.

-The University of Arizona Cancer Center is an NCI (National Cancer Institute) designated Comprehensive Cancer Center, one of 41 in the country.

-A requirement of being an NCI designated Comprehensive Cancer Center is to have a biorepository, in other words a big tissue bank.

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-We will also take a small syringe of blood, this is done in pre-op when your IV is started, thus no additional needle stick will be required.

-You do not benefit from being in the study

-There is NO cost to you for being in the study

-Frequently, the participants will question to agreeing to follow up check mark box. Stress they have the right to refuse this if they choose or to be removed from participating at any time.

-After signing, offer to make them a copy of the signed consent.

Rev		Effective Date	
1/03/2020 Ver 7	**SOP for the UACC Biospecimen Repository**	08-01-2006	McDaniel

1. PURPOSE & SCOPE

To ensure that information necessary to meet the goals and objectives of the Study is managed effectively and to ensure that the privacy of subjects is protected and the confidentiality and security of the data is maintained.

2. DEFINITIONS

- Institutional Review Board (IRB): Applicable guidelines, certifications, and Verification of Training (VoTF) requirements are administered by the University of Arizona (UA) Human Subjects Protection Program, Office for the Responsible Research Conduct of Research, http://rgw.arizona.edu/compliance/human-subjects-protection-program
- TACMASR: Tissue Acquisition and Cellular/Molecular Analysis Shared Resource is the "core" lab that is responsible for management of the UACC Biospecimen Repository (Tumor Bank). TACMASR also provides histological and pathological services to UACC investigators. http://uacc.arizona.edu/research/shared-resources/tacmasr
- TissueMetrix (AIM) is the biospecimen information management system selected by the University of Arizona Cancer Center to manage and track several existing biorepositories of tissues and biospecimens. As such, the UACC Biospecimen Repository does not have oversight over TissueMetrix policy and procedures.

3. REFERENCES

Project Authority: University of Arizona Cancer Center Biospecimen Repository IRB Project #06-0609-04
Setsuko K. Chambers, MD, Principal Investigator

- SOP 400-2 Overview of Study Processes
- SOP 400-5 Steps to Enroll Subjects and Bank Biospecimens
- SOP 400-6 Monitoring Plan and Tools for Quality Assurance
- SOP 400-10 Tissue Acquisition and Processing
- SOP 400-11 Processing Blood Samples
- TissueMetrix Flowchart for Labeling and Workflow
- TissueMetrix Collection Event Log Form and Sample-type Log Forms (Blank Forms)

4. RESPONSIBILITIES

4.1. TACMASR personnel who have access to subject samples and information as part of their job duties are responsible for following the procedures in the SOP in order to protect subject privacy and confidentiality at all times

4.2. The Lab Manager and Database Administrator are responsible for training and oversight of personnel who are required to access, enter and manage electronic data in the performance of their job duties

4.3. Deviations are to be documented on the Collection Event Form

4.4. Unexpected events are to be reported to the Lab Manager, and TACMASR Director

4.5. The Biospecimen Oversight Committee approves final distribution of specimens and information.

5. STUDY REQUIREMENTS TO OBTAIN ACCESS TO INFORMATION

5.1. TACMASR personnel who may access subject information includes the PI, TACMASR Director and Biorepository staff, who are required to manage confidential electronic information in the performance of their job duties.

5.2. All TACMASR personnel must be listed on the VoTF.

5.3. Patient information necessary to this Study includes access to the OR schedule, patient identifiers, demographics, pathology reports and clinical information

5.4. Biorepository personnel who are required to manage confidential electronic information must obtain the appropriate training and login/password access, which includes the University of Arizona Health Center and the TissueMetrix information system

5.5. TissueMetrix is the biospecimen management software that UACC uses to track biospecimens, from acquisition to long term storage. TissueMetrix is a critical tool in UACC's efforts to effectively manage, track, and document information to ensure patient privacy

6. PROCEDURES TO OBTAIN ACCESS TO MEDICAL RECORDS

6.1. Banner University Medical Center requires anyone with permission to access this database to take a privacy and training course before a user ID is given

6.2. Banner University Medical Center medical records and OR schedule are accessed through the ~~EPIC~~ Cerner database management information system.

6.3. CoPath and CERNER are the information systems used by Banner University Medical Center to access pathology reports

6.4. Contact the Banner University Medical Center Help Desk at 694-4357 to install and assist with CERNER

6.5. To install CITRIX, go to https://www.umcaz.edu/citrix and then to the last link on the page

7. PROCEDURES TO OBTAIN ACCESS TO TISSUE METRIX

7.1. The TissueMetrix program is encoded, requires a login and password, and allows for restricted access to identifiable information (such as subject name, MRN, DOB, etc.)

7.2. Data is searchable and reports may be created

7.3. The Database Administrator sets up individual accounts, trains authorized users, and provides ongoing database maintenance and troubleshooting

7.4. TissueMetrix manages study information including: Subject information obtained from the consent, information recorded on the Collection Event Log, the Sample-type logs, and pathology reports

7.5. All Subjects and specimens entered from January 1, 2011 and going forward, are assigned codes generated for entry into TissueMetrix

7.6. Auditing and entering of legacy data is ongoing.

7.7. The system allows for restricted access to aggregate information through the “Storefront” application

7.8. The Storefront displays a real time inventory of the number and types of biospecimens that are available to UACC investigators for use in translational research via the University of Arizona Cancer Center’s website.

7.8.1. TACMASR staff and students are the personnel TACMASR who enter information into TissueMetrix.

8. STUDY INFORMATION ENTERED INTO TISSUE METRIX

8.1. The Lab Manager and students enter information into TissueMetrix gathered from Collection Event Logs, Pathology reports, electronic patient notes and from tissue slides that are prepared by the Repository histology personnel (H&E slides made from each frozen and paraffin tissue block entered into the bank)

8.2. From the specimen collection process, information obtained and entered into TissueMetrix includes: name, medical record number (MRN), visit number, gender, date of birth, age at time of surgery, date of surgery (DOS), attending physician, surgeon, types and volumes of specimens collected, and the time of specimen collection and processing

8.3. Information that is obtained and entered into TissueMetrix from the pathology specimen slides and the pathology report include: Tissue stage, type, size, grade, and anatomical location

8.4. Information that may be obtained for clinical annotation purposes by accessing the medical records includes: medical history, family history, race, past and current medications, environmental risk exposure

8.5. Specimen storage location information is entered into searchable fields to locate available specimens

9. THE TISSUE METRIX INFORMATION LABELING SYSTEM

9.1. The Database Administrator maintains a secure database of all accession numbers assigned.

9.2. All identifiers are unique at each level (donor, event, specimen) in order to in order to prevent the generation of duplicate identification numbers

9.3. A Participant is considered "enrolled" in the Study when informed consent authorization document have been received and verified by the Tumor Bank Coordinator

9.4. Each Participant will have only one unique, randomly generated "PTID" number, but may have multiple collection Events (different surgical procedures on different dates). Each Collection Event may have multiple, individual biospecimens collected as part of the same event (serum, plasma, tissue, etc.)

9.5. Each Participant's PTID number is randomly generated and uniquely assigned to each consented participant. The PTID number is formatted as follows: a three-character alpha prefix, "AZR", identifies the Biorepository in TissueMetrix. AZR is followed by a five digit numerical identifier: AZRXXXXX

9.6. Each Collection Event for each Participant receives a Collection Event Log form, and each Event is assigned a sequential identification number, which is formatted as follows:

> A one character alpha prefix, "R", identifies the Biorepository, followed by a two digit numerical identifier designating the year of collection, then a four digit numerical identifier which is sequentially generated and uniquely assigned to each collection event" "R16XXXX"

9.7. Each Individual specimen from each Collection Event is identified by adding two more numerical digits to the Collection Event identifier, thusly: R16XXXX01, 02, etc.

9.8. Duplicate barcode labels with the PTID number are printed: one label is affixed to the consent document and the other is affixed to the Participant's Collection Event Log form

9.9. A Barcode label with the Collection Event number is printed and affixed to the (paper) Collection Event Form.

9.10. Duplicate Specimen labels are printed for each individual biospecimen: one is a cryo label and is affixed to the specimen (cryovial, frozen tissue bag, paraffin cassette, etc.). The second label is paper and is affixed to the appropriate Sample-Type Box Log form in order to assign storage locations for each individual specimen, e.g., Box locations for each aliquot of serum or piece of tissue, or drawer locations for each paraffin block.

9.11. The Database Administrator prints and distributes identification labels and barcode labels for the Study, and maintains master copies of all log forms used in the Study.

10. DOCUMENT FILING

10.1.1. The Tissue Bank Coordinator files study documents in 3-ring binders; each Participant's file includes properly signed consent authorization forms, Collection Event Log, pathology surgical reports, and pathology specimen review forms (H&E slide reviews).

10.2. Documents are filed in UACC in the TACMASR office and records room, Room 0914 and 0903, which has restricted access

10.3. The Lab Manager verifies that only authorized personnel have access to this room

11. DISTRIBUTION OF INFORMATION

11.1. Researchers interested in utilizing banked specimens must first obtain IRB approval and Biospecimen Oversight Committee approval (Refer to SOP 400-7 *Utilization of Biospecimens*)

11.2. Determination of Human Research must be signed and submitted to TACMASR by an investigator who is requesting de-identified biospecimens with no access to patient identifiers

11.3. The Biorepository Operations Manager, Biorepository Coordinator or Study personnel may interrogate the TissueMetrix database to identify potential, available specimens that meet the investigator's criteria

11.4. TACMASR research personnel who are preparing specimens retrieve the banked materials and work with the Pathologist to de-identify and prepare the material and associated information for use by the investigator

11.5. The Biorepository Coordinator or Study personnel documents the status of any remaining material that would still be available in the Biorepository

Tissue Acquisition and Processing

SOP 400-10

9/8/2017
Version 007

	Signature	Date
Originator/Reviser:	_____	_____
Reviewed/Approved by:	_____	_____

Rev		Effective Date	
1/19/19 Ver 8	**SOP for the UACC Biospecimen Repository**	10-20-2016	Kepler

1. PURPOSE & SCOPE

To define procedures relating to the collection, processing and transport of biospecimens from Banner University Medical Center operating room (OR) to the UACC Biorepository laboratory. Procedures are designed to minimize variability and optimize the collection and preservation of high quality biospecimens.

2. DEFINITIONS

TissueMetrix (AIM) is the biospecimen information management system administered through the University of Arizona Cancer Center.

3. REFERENCES

- SOP 400-5 Steps to Enroll Subjects and Bank Biospecimens
- SOP 400-9 Information Management
- SOP 400-XX Tissue processing & Embedding
- SOP 400-XX H & E Staining
- "Bloodborne Pathogen Exposure Control Plan." University of Arizona. Revised August 2003. Reference 29 CFR 1910.1030.
- SOP 400-10 Appendix I – Collection Contacts
- SOP 400-10 Appendix II – Transport cart checklist

4. RESPONSIBILITIES

4.1. TACMASR Laboratory research personnel who are preparing samples for banking are responsible for following the procedures in the SOP and confirming that each step has been properly executed.

4.2. Deviations are to be documented on the Collection Event Form.

4.3. Unexpected events are to be reported to the Coordinator, Lab Manager, and TACMASR Director.

4.4. The Coordinator and Lab Manager are responsible for training and daily oversight of personnel who perform tissue preparation procedures**.**

5. SAFETY AND CAUTIONARY NOTES

5.1. Universal precautions and sterile technique should be followed. At minimum this should include the use of gloves, eye protection and lab coat.

5.2. All equipment and surfaces, forceps, dissecting board, that come in contact with tissue must be decontaminated with Converge upon the completion of tissue processing.

5.3. Used scalpels should be disposed of in the sharps container located on the transport cart.

5.4. Scrubs must be worn to enter the OR. Before entering the OR suite ~~Once in the~~ ~~frozen section room~~ a disposable surgical cap, mask and shoe covers are available to don before entering the actual OR suite.

5.5. Acquisition of tissue is prioritized as follows:

5.5.1. Frozen OCT blocks,

5.5.2. Other institutional studies

5.5.3. Formalin-fixed paraffin embedded (FFPE) tissue blocks.

5.6. Tissue can only be grossed and handed off to Biorepository personnel for banking by a pathology assistant, pathology resident or pathology attending.

5.7. Tissue should be snap frozen as soon as possible to preserve the morphology, protein biomarkers and RNA.
5.8. Each tissue specimen is labeled with the collection bar code.
5.9. Complete the corresponding collection event log.

6. MATERIALS AND EQUIPMENT

6.1. TissueMetrix Collection Event form and the sequential Collection ID barcodes that are linked to the PTID. Refer to SOP 400-9: Information Management for details regarding the TissueMetrix labeling system.
6.2. Collection Cart
6.3. Lead marking pencil, permanent marking pen, forceps (VWR or equivalent)
6.4. Metal Dewar bowl and stainless steel beaker
6.5. Dewar flask filled liquid nitrogen
6.6. Dry Ice in a Styrofoam box
6.7. Isopentane (2-methyl-butane) VWR JTQ223-8
6.8. OCT compound and cryomolds (Leica)
6.9. Specimen bags (Bitran)
6.10. 1.5ml cryovials (USA Scientific)
6.11. RNAlater (Ambion)
6.12. Igloo cooler
6.13. Syrofoam box with wet ice

7. COLLECTION PROCEDURE

7.1. Patients are consented in clinic at their pre-surgery appointment. Clinic staff will notify the Biorepository of consented patients with their surgery date. Time of surgery can be determined by viewing the OR schedule within ~~EPIC.~~ Cerner

7.2. Obtain a new Collection Event Form, Biospecimen Labels and current Box Logs in preparation for a potential collection. Refer to SOP 400-9 that describes the use of the collection event form.

7.3. Prepare the transport cart. Fill a Styrofoam box with dry ice, located in the cryobox in room 0915. Fill the liquid nitrogen Dewar with liquid nitrogen, located in room 0915. Ensure the working solution of Isopentane, kept in a glass bottle on the freezing cart, has at least 150ml of solution in it. It can be replenished when low from the stock bottle of Isopentane stored in the flammables cabinet under the fume hood in room 0915.

7.4. Verify the cart contains the rest of the items needed for collection. See Appendix II for details.

7.4.1. Monitor when a surgical procedure has started from the OR status board within ~~EPIC~~.Cerner Once thesurgery has started, telephone into the appropriate OR room to alert the circulating nurse know the patient is consented for tissue collection. When calling in, state your name, that you are from Tumor Bank, the physician's name, and the patient's name. Give the OR nurse the pager number~~, 8577~~.531-5974

7.5. When the OR pages that the specimen is ready for pickup, note the time of page on the collection event form. Call the OR to verify that you received their page, the tissue has been excised and you will be in to pick it up.

7.6. Proceed with the transport cart to the frozen section room. Once inside there, prepare to enter the OR by donning protective apparel in the following order: surgical cap, surgical mask and shoe covers. Enter the OR suite and go directly to the specified OR room.

7.7. Cautiously enter the operating room. Do not touch anything sterile. Stand well back from the operation in progress, as close to the perimeter wall as possible. Locate the circulating nurse. He or she will hand over the tissue in a biospecimen container. Verify the label on the container is the correct patient and specimen.

7.8. Exit the OR suite and return to the frozen section room. Record the time of tissue collection on the collection event form.

7.9. If the surgeon has called for a frozen section, a pathology resident will have been called by the OR nurse. In this case, tissue for banking may be obtained there in the frozen section room AFTER the frozen sectioning is completed and the Pathologist or Resident hands off the tissue designated for banking. If no frozen section has been called for, place the specimen container in the bottom drawer of the transport cart and proceed to the morgue for grossing.

7.10. Pathology will determine if there is enough tumor for banking. The size of the specimen provided to the biorepository depends on the amount and specimens banked. Tissue pieces should be no larger than 5mm thick by 20mm wide for optimal fixation (snap freezing or formalin).

~~7.11.~~ Tissue specimens are prioritized as follows: OCT frozen tissue, FFPE, Fresh frozen. In addition to the tumor specimen, a matching normal piece of tissue can be requested if possible.

7.12. As soon as the tissue specimens have been received (in the Frozen Section Room or the Morgue), proceed to process using materials on the transport cart. Place the piece of tissue on a small section of modeling wax and lay that on the dissecting board. Using a sterile scalpel, cut the tissue into sections if it is large enough to divide for freezing and fixing in formalin. If not freeze the entire piece in OCT.

Procedure to freeze tissue in OCT

- Pre-chill the isopentane by filling the metal dewar bowl half way with liquid nitrogen. Pour isopentane into the metal beaker so it's one third full. Set the metal beaker into the dewar bowl. Isopentane begins to thicken after 1-2 minutes forming a syrupy consistency (approximately 150 °C. It will turn white on the bottom of the beaker when chilled and ready for freezing.
- Label one cryomold and one Bitran Ziploc specimen bag for each tissue specimen banked. Use alcohol resistant marker pens to label. Place the specimen bag on dry ice for pre chilling.
- Bend the labeled end of the cryomold so that it can be picked up with the forceps.
- Place a small amount of OCT in the bottom of the cryomold then place the tissue section in the cryomold.
- Fill the cryomold with OCT compound avoiding trapping air bubbles in the OCT.
- Gently lower the tissue into the chilled isopentane.
- Leave the cryomold in the isopentane for approximately 30 seconds until frozen.
- Remove and place on dry ice while popping the frozen block out of the cryomold. Place the frozen OCT block in the pre-labeled and pre chilled specimen bag. Keep surrounded by dry ice for transport back to the biorepository and transfer to a -80 °C freezer.
- Record the time frozen on the collection event form as well as the sample type and tissue.
- Dispose of the scalpel in the biohazard container on the transport cart. The used piece of dental wax should be disposed of in the biohazard container in pathology.
- Pour the remaining liquid nitrogen back into the liquid nitrogen dewar. Pour the isopentane back into the glass bottle.
- Spray the forceps and dissecting board with Converge spray located in pathology.

Procedure to collect tissue in the new Banner hospital

- **Prepare the Igloo cooler , stryrofoam box with wet ice, cryomolds, marker, OCT compound**
- **When paged, proceed to Banner Tower 1. Drop the Igloo cooler off in pathology.**
- **Outside of pathology and right before entering the OR suite are masks, hat and shoes covers. Don these.**

- **Enter the OR suite and locate the OR of the surgery.**
- **Enter the OR and retrieve the specimen container from the circulating nurse at the nurses station.**
- **Take the container & tissue back to pathology for grossing.**
- **Once in pathology, don a protective coat and eyeware.**

Procedure to fix tissue in formalin for paraffin embedding

- Using a lead pencil, label a tissue cassette with the collection number on the top and the tissue type on the right hand side.
- Place the piece of tissue in the cassette and lock the cassette lid in place.
- Fill a specimen cup 2/3 with 10% neutral buffered formalin and place the tissue cassette into it.
- Record the time and date placed in formalin on the lid of the collection cup and on the collection event form.
- Place a pink Buffered formalin sticker on the side of the specimen cup. These are located in pathology by the formalin container.
- Specimens should be fixed in formalin at room temperature between 5-24 hours. Do not leave in formalin over 24 hours, after this time the tissue is overfixed and the DNA will begin to fragment.
- Transfer the cassette to 70% ethanol and store in the specimen refrigerator in the biorepository lab. Specimens can be stored in alcohol for up to 7 days before processing.
- Record the date and time transferred from formalin to ethanol on the collection event log.

Procedure to preserve tissue in RNAlater

- 1.5ml cryovials are sterilely filled with 1.0ml of RNAlater and stored on the collection cart.
- After obtaining tissue from pathology, drop a piece of tumor tissue no larger than 5mm x 5mm into a cryovial labeled with the collection number, tissue type and date.
- Repeat the same process with normal tissue.
- Store the tissue on the cart until return to the TACMASR laboratory.
- Once in the laboratory store the RNAlater vials containing the tissue in the small refrigerator located on the counter next to the entrance door.
- Tissue should remain in the refrigerator for a minimum of 24hours and no longer than 5 days.
- After that time transfer the vials to -80 Freezer F in 0963 for long term storage.
- Record the collection number on the RNAlater log hanging on door of Freezer F.
- Record the box and slot of vials placed into the -80 on the collection event log.

Biorepository OPERATING PROCEDURE 400-10 Appendix I

COLLECTION CONTACTS

OR Front Desk	**694-6120**
Pre-op	**694-2220**
OR Rooms	~~**964-72**~~694-31- - (last two digits of OR room#)
~~**OR Room 17 (exception)**~~	~~**964-5858**~~
~~**Frozen Section room (1648)**~~	~~**694-6480**~~
~~**Morgue**~~ **Pathology**	**694-6564**
~~**Paging system**~~	~~**694-4480**~~
Biorepository Pager#	~~**8577**~~**531-5974**

Biorepository Operating Procedure 400-10 Appendix II

TRANSPORTATION CART CHECKLIST

The following is a list of items that should be routinely stocked and stored on the cart**:**

- Forceps
- Modeling wax
- Dissecting board
- Permanent, alcohol resistant, Marker/Sharpie
- Lead Pencil
- Biotran specimen bags
- Shallow Dewar Bowl
- Stainless steel beaker
- Liquid Nitrogen Dewar Flask
- Specimen cups
- Scalpels
- Biohazard Sharps container
- RNAlater filled cryovials
- OCT cryomolds (standard size)
- OCT Embedding media
- Tissue Cassettes
- Gloves

The following items must be added to the cart on the day of collection:

- Dry Ice
- Liquid Nitrogen

- Isopentane

Tissue Acquisition and Processing

SOP 400-10

9/8/2017
Version 007

	Signature	Date
Originator/Reviser:	_____	_____
Reviewed/Approved by:	_____	_____

Rev		Effective Date	
1/19/19 Ver 8	**SOP for the UACC Biospecimen Repository**	10-20-2016	Kepler

1. PURPOSE & SCOPE

To define procedures relating to the collection, processing and transport of biospecimens from Banner University Medical Center operating room (OR) to the UACC Biorepository laboratory. Procedures are designed to minimize variability and optimize the collection and preservation of high quality biospecimens.

2. DEFINITIONS

TissueMetrix (AIM) is the biospecimen information management system administered through the University of Arizona Cancer Center.

3. REFERENCES

- SOP 400-5 Steps to Enroll Subjects and Bank Biospecimens
- SOP 400-9 Information Management
- SOP 400-XX Tissue processing & Embedding
- SOP 400-XX H & E Staining
- "Bloodborne Pathogen Exposure Control Plan." University of Arizona. Revised August 2003. Reference 29 CFR 1910.1030.
- SOP 400-10 Appendix I – Collection Contacts
- SOP 400-10 Appendix II – Transport cart checklist

4. RESPONSIBILITIES

4.1. TACMASR Laboratory research personnel who are preparing samples for banking are responsible for following the procedures in the SOP and confirming that each step has been properly executed.

4.2. Deviations are to be documented on the Collection Event Form.

4.3. Unexpected events are to be reported to the Coordinator, Lab Manager, and TACMASR Director.

4.4. The Coordinator and Lab Manager are responsible for training and daily oversight of personnel who perform tissue preparation procedures**.**

5. SAFETY AND CAUTIONARY NOTES

5.1. Universal precautions and sterile technique should be followed. At minimum this should include the use of gloves, eye protection and lab coat.

5.2. All equipment and surfaces, forceps, dissecting board, that come in contact with tissue must be decontaminated with Converge upon the completion of tissue processing.

5.3. Used scalpels should be disposed of in the sharps container located on the transport cart.

5.4. Scrubs must be worn to enter the OR. Before entering the OR suite ~~Once in the~~ ~~frozen section room~~ a disposable surgical cap, mask and shoe covers are available to don before entering the actual OR suite.

5.5. Acquisition of tissue is prioritized as follows:

5.5.1. Frozen OCT blocks,

5.5.2. Other institutional studies

5.5.3. Formalin-fixed paraffin embedded (FFPE) tissue blocks.

5.6. Tissue can only be grossed and handed off to Biorepository personnel for banking by a pathology assistant, pathology resident or pathology attending.

5.7. Tissue should be snap frozen as soon as possible to preserve the morphology, protein biomarkers and RNA.
5.8. Each tissue specimen is labeled with the collection bar code.
5.9. Complete the corresponding collection event log.

6. MATERIALS AND EQUIPMENT

6.1. TissueMetrix Collection Event form and the sequential Collection ID barcodes that are linked to the PTID. Refer to SOP 400-9: Information Management for details regarding the TissueMetrix labeling system.
6.2. Collection Cart
6.3. Lead marking pencil, permanent marking pen, forceps (VWR or equivalent)
6.4. Metal Dewar bowl and stainless steel beaker
6.5. Dewar flask filled liquid nitrogen
6.6. Dry Ice in a Styrofoam box
6.7. Isopentane (2-methyl-butane) VWR JTQ223-8
6.8. OCT compound and cryomolds (Leica)
6.9. Specimen bags (Bitran)
6.10. 1.5ml cryovials (USA Scientific)
6.11. RNAlater (Ambion)
6.12. Igloo cooler
6.13. Syrofoam box with wet ice

7. COLLECTION PROCEDURE

7.1. Patients are consented in clinic at their pre-surgery appointment. Clinic staff will notify the Biorepository of consented patients with their surgery date. Time of surgery can be determined by viewing the OR schedule within ~~EPIC.~~ Cerner

7.2. Obtain a new Collection Event Form, Biospecimen Labels and current Box Logs in preparation for a potential collection. Refer to SOP 400-9 that describes the use of the collection event form.

7.3. Prepare the transport cart. Fill a Styrofoam box with dry ice, located in the cryobox in room 0915. Fill the liquid nitrogen Dewar with liquid nitrogen, located in room 0915. Ensure the working solution of Isopentane, kept in a glass bottle on the freezing cart, has at least 150ml of solution in it. It can be replenished when low from the stock bottle of Isopentane stored in the flammables cabinet under the fume hood in room 0915.

7.4. Verify the cart contains the rest of the items needed for collection. See Appendix II for details.

7.4.1. Monitor when a surgical procedure has started from the OR status board within ~~EPIC~~.Cerner Once thesurgery has started, telephone into the appropriate OR room to alert the circulating nurse know the patient is consented for tissue collection. When calling in, state your name, that you are from Tumor Bank, the physician's name, and the patient's name. Give the OR nurse the pager number~~, 8577~~.531-5974

7.5. When the OR pages that the specimen is ready for pickup, note the time of page on the collection event form. Call the OR to verify that you received their page, the tissue has been excised and you will be in to pick it up.

7.6. Proceed with the transport cart to the frozen section room. Once inside there, prepare to enter the OR by donning protective apparel in the following order: surgical cap, surgical mask and shoe covers. Enter the OR suite and go directly to the specified OR room.

7.7. Cautiously enter the operating room. Do not touch anything sterile. Stand well back from the operation in progress, as close to the perimeter wall as possible. Locate the circulating nurse. He or she will hand over the tissue in a biospecimen container. Verify the label on the container is the correct patient and specimen.

7.8. Exit the OR suite and return to the frozen section room. Record the time of tissue collection on the collection event form.

7.9. If the surgeon has called for a frozen section, a pathology resident will have been called by the OR nurse. In this case, tissue for banking may be obtained there in the frozen section room AFTER the frozen sectioning is completed and the Pathologist or Resident hands off the tissue designated for banking. If no frozen section has been called for, place the specimen container in the bottom drawer of the transport cart and proceed to the morgue for grossing.

7.10. Pathology will determine if there is enough tumor for banking. The size of the specimen provided to the biorepository depends on the amount and specimens banked. Tissue pieces should be no larger than 5mm thick by 20mm wide for optimal fixation (snap freezing or formalin).

~~7.11.~~ Tissue specimens are prioritized as follows: OCT frozen tissue, FFPE, Fresh frozen. In addition to the tumor specimen, a matching normal piece of tissue can be requested if possible.

7.12. As soon as the tissue specimens have been received (in the Frozen Section Room or the Morgue), proceed to process using materials on the transport cart. Place the piece of tissue on a small section of modeling wax and lay that on the dissecting board. Using a sterile scalpel, cut the tissue into sections if it is large enough to divide for freezing and fixing in formalin. If not freeze the entire piece in OCT.

Procedure to freeze tissue in OCT

- Pre-chill the isopentane by filling the metal dewar bowl half way with liquid nitrogen. Pour isopentane into the metal beaker so it's one third full. Set the metal beaker into the dewar bowl. Isopentane begins to thicken after 1-2 minutes forming a syrupy consistency (approximately 150 °C. It will turn white on the bottom of the beaker when chilled and ready for freezing.
- Label one cryomold and one Bitran Ziploc specimen bag for each tissue specimen banked. Use alcohol resistant marker pens to label. Place the specimen bag on dry ice for pre chilling.
- Bend the labeled end of the cryomold so that it can be picked up with the forceps.
- Place a small amount of OCT in the bottom of the cryomold then place the tissue section in the cryomold.
- Fill the cryomold with OCT compound avoiding trapping air bubbles in the OCT.
- Gently lower the tissue into the chilled isopentane.
- Leave the cryomold in the isopentane for approximately 30 seconds until frozen.
- Remove and place on dry ice while popping the frozen block out of the cryomold. Place the frozen OCT block in the pre-labeled and pre chilled specimen bag. Keep surrounded by dry ice for transport back to the biorepository and transfer to a -80 °C freezer.
- Record the time frozen on the collection event form as well as the sample type and tissue.
- Dispose of the scalpel in the biohazard container on the transport cart. The used piece of dental wax should be disposed of in the biohazard container in pathology.
- Pour the remaining liquid nitrogen back into the liquid nitrogen dewar. Pour the isopentane back into the glass bottle.
- Spray the forceps and dissecting board with Converge spray located in pathology.

Procedure to collect tissue in the new Banner hospital

- **Prepare the Igloo cooler , stryrofoam box with wet ice, cryomolds, marker, OCT compound**
- **When paged, proceed to Banner Tower 1. Drop the Igloo cooler off in pathology.**

- **Outside of pathology and right before entering the OR suite are masks, hat and shoes covers. Don these.**
- **Enter the OR suite and locate the OR of the surgery.**
- **Enter the OR and retrieve the specimen container from the circulating nurse at the nurses station.**
- **Take the container & tissue back to pathology for grossing.**
- **Once in pathology, don a protective coat and eyeware. Record the time the tissue was collected.**
- **Pathology personnel will gross the tissue and determine if there is enough tumor for banking. The size of the specimen provided to the biorepository should be no larger than 5mm thick by 20mm wide for optimal fixation.**
- **Tissue specimens are prioritized as follows: OCT frozen, FFPE and then Fresh frozen.**
- **If possible obtain a matching piece of normal tissue**
- **The piece of tissue to be preserved as OCT frozen is placed into the cryomold, filled with OCT compound, then placed on the wet ice in the Igloo cooler.**
- **Return to the Cancer Center Biorepository lab and snap freeze the OCT preserved sample using the supplies on the transportation cart.**

Procedure to fix tissue in formalin for paraffin embedding

- Using a lead pencil, label a tissue cassette with the collection number on the top and the tissue type on the right hand side.
- Place the piece of tissue in the cassette and lock the cassette lid in place.
- Fill a specimen cup 2/3 with 10% neutral buffered formalin and place the tissue cassette into it.
- Record the time and date placed in formalin on the lid of the collection cup and on the collection event form.
- Place a pink Buffered formalin sticker on the side of the specimen cup. These are located in pathology by the formalin container.
- Specimens should be fixed in formalin at room temperature between 5-24 hours. Do not leave in formalin over 24 hours, after this time the tissue is overfixed and the DNA will begin to fragment.
- Transfer the cassette to 70% ethanol and store in the specimen refrigerator in the biorepository lab. Specimens can be stored in alcohol for up to 7 days before processing.
- Record the date and time transferred from formalin to ethanol on the collection event log.

Procedure to preserve tissue in RNAlater

- 1.5ml cryovials are sterilely filled with 1.0ml of RNAlater and stored on the collection cart.
- After obtaining tissue from pathology, drop a piece of tumor tissue no larger than 5mm x 5mm into a cryovial labeled with the collection number, tissue type and date.
- Repeat the same process with normal tissue.
- Store the tissue on the cart until return to the TACMASR laboratory.
- Once in the laboratory store the RNAlater vials containing the tissue in the small refrigerator located on the counter next to the entrance door.
- Tissue should remain in the refrigerator for a minimum of 24hours and no longer than 5 days.
- After that time transfer the vials to -80 Freezer F in 0963 for long term storage.
- Record the collection number on the RNAlater log hanging on door of Freezer F.
- Record the box and slot of vials placed into the -80 on the collection event log.

Biorepository OPERATING PROCEDURE 400-10 Appendix I

COLLECTION CONTACTS

OR Front Desk	**694-6120**
Pre-op	**694-2220**
OR Rooms	~~**964-72**~~694-31- - (last two digits of OR room#)
~~**OR Room 17 (exception)**~~	~~**964-5858**~~
~~**Frozen Section room (1648)**~~	~~**694-6480**~~
~~**Morgue**~~ **Pathology**	**694-6564**
~~**Paging system**~~	~~**694-4480**~~
Biorepository Pager#	~~**8577**~~ **531-5974**

Biorepository Operating Procedure 400-10 Appendix II

TRANSPORTATION CART CHECKLIST

The following is a list of items that should be routinely stocked and stored on the cart**:**

- Forceps
- Modeling wax
- Dissecting board
- Permanent, alcohol resistant, Marker/Sharpie
- Lead Pencil
- Biotran specimen bags
- Shallow Dewar Bowl
- Stainless steel beaker
- Liquid Nitrogen Dewar Flask
- Specimen cups
- Scalpels
- Biohazard Sharps container
- RNAlater filled cryovials

- OCT cryomolds (standard size)
- OCT Embedding media
- Tissue Cassettes
- Gloves

The following items must be added to the cart on the day of collection:

- Dry Ice
- Liquid Nitrogen
- Isopentane

IGLOO COOLER CHECKLIST

- Stryrofoam box with wet ice
- Cryomolds
- OCT embedding media
- Tissue Cassettes
- Specimen container
- Permenant marking pen
- Lead pencil

SOP # **400-11** Subject **Preparation of Blood Samples** Sheet **1** of **3**

Rev		Effective Date	Author
9/08/2017 Ver 6	SOP for the UACC Biospecimen Repository	Jan 2008	Kepler

1. PURPOSE & SCOPE

To ensure proper collection, handling and preservation of blood samples which are entered into the UACC Biospecimen Repository.

2. DEFINITIONS

TissueMetrix (AIM) is the biospecimen information management system administered through the University of Arizona Cancer Center.

3. REFERENCES

- Desktop SOP 400-5: Steps to Enroll Subjects and Bank Biospecimens
- Desktop SOP 400-9: Information Management

4. RESPONSIBILITIES

4.1. TACMASR Laboratory research personnel who preparing samples for banking are responsible for following the procedures in the SOP and confirming that each step has been executed properly
4.2. Deviations are to be documented on the Collection Event Form
4.3. Unexpected events are to be reported to the Lab Manager or Lab Director

5. SAFETY AND CAUTIONARY NOTES

5.1. Universal precautions and sterile technique should be followed. At minimum this should include the use of gloves, eye protection and lab coat. All processing of specimens should take place under a laminar flow hood.
5.2. Any spills or drops of blood should be immediately cleaned up by first spraying the spill area with 10% bleach, followed by 70% ethanol.
5.3. All spent tubes and pipet tips are to be discarded in a red biohazard container.
5.4. Blood should be allowed to sit for 30 minutes to allow for the serum red top tube to completely clot.
5.5. After 30 minutes blood can be processed. If processing is to be delayed, store blood in the specimen refrigerator in room 0915. If needed blood can be left overnight in the refrigerator and processed the following morning. This is not preferred however.
5.6. Complete the corresponding collection event form.

6. MATERIALS AND EQUIPMENT

6.1. Serum Vacutainer tubes, red top, plastic, 6 ml tubes: BD367863 (VWR).
6.2. K2 EDTA Vacutainer tubes, lavender top, plastic, 6 mls, BD367815 (VWR).
6.3. 2.0ml Round Bottom, self-standing, cryogenic vials: VWR #89094-810.
6.4. Blue cryovial cap inserts.
6.5. Red cryovial cap inserts VWR
6.6. Yellow cryovial cap insert VWR
6.7. Saf-T-Zip bag 6 x 9” Pack of 100 CAT# 11217-516 (Fisher).
6.8. P-1000 Pipettors and sterile P-1000 filter pipette tips.
6.9. Sorvall Legend RT Tabletop refrigerated centrifuge located in Room 3957.
6.10. Biohazard containers

7. BLOOD COLLECTION

7.1. Obtain the TissueMetrix Collection Event form and the sequential Specimen ID barcodes that are linked to the PTID. Refer to *SOP 400-9: Information Management* for details regarding the TissueMetrix labeling system.
7.2. The Biorepository requests two 6ml tubes of blood from consented study participants, K2 EDTA lavender top tube and a red top serum tube.
7.3. Pre-op is alerted by Biorepository personnel the evening before of the next day's consented patients.
7.4. The Biorepository is responsible for stocking Pre-op with blood collection kits. Each kit (biospecimen bag) includes one red top tube, one lavender top tube and a Tissue Bank index card with the Biorepository phone number (626-7319) and pager number ~~8577~~ 531-5974 on it.
7.5. When paged, Biorepository personnel proceeds to Pre-op and retrieve the blood. Pre-op puts the blood in a basket labeled tumor bank on the main desk.
7.6. Record the patient information on the collection event form: name, MRN, DOS, DOB, sex, race/ethnicity, attending physician.
7.7. Record the time and date of blood collection as well as the date and time of processing.

8. WHOLE BLOOD PROCESSING

Refer to *SOP 400-9 Information Management,* for details regarding the TissueMetrix labeling system.

8.1. With a marking pen, label 3 cryovials with sequential collection ID numbers designated in the collection kit. The collection number suffix for whole blood is 01, 02, and 03. For example R160XXX 01, R160XXX 02, R160XXX 03.
8.2. Place the corresponding cryolabel on the tube so that it does not cover up the marking pen labeling.
8.3. Insert BLUE colored cryocap inserts into the top of each cryovial used.
8.4. Invert the EDTA tube 10 times to insure proper mixing of blood.
8.5. Carefully remove the cap from the Vacutainer tube and using a P-1000 pipet and a P-1000 filter tip, transfer 400ul of whole blood to each labeled cryovial.
8.6. Dispose of used tip in the biohazard container located in the back of the laminar flow hood.
8.7. Place the cap back on the Vacutainer tube.

9. PLASMA PROCESSING

9.1 Centrifuge the remaining blood in the EDTA tube at 3000 RPM, 4°C, for 10 minutes. The Sovall Legend RT tabletop refrigerated centrifuge is located in room 0915. Tubes should be placed in biosafety aerosol canister and each canister balanced prior to spinning.
9.2 With a marking pen, label two cryovials with sequential collection ID numbers designated in the collection kit. The collection number suffix for EDTA plasma is 06, and 07. For example, R160XXX 06, R160XXX 07.
9.3 Insert yellow colored cryocap inserts into the top of each cryovial used.
9.4 Place the corresponding cryovial label on the tube so that it does not cover up the marking pen labeling.
9.5 Carefully remove the cap from the Vacutainer tube and using a P-1000 pipet with a filter tip, transfer 1000ul of plasma into each pre labeled cryovial. Depending on the amount of plasma in the tube, aliquots may be less than 1000ul.
9.6 Place the cap back on the Vacutainer tube and dispose of it and the pipet tip in the biohazard container located in the back of the laminar flow hood.

10. SERUM PROCESSING

10.1 After red top Vacutainer has been allowed to clot for 30 minutes, centrifuge it at 3000 RPM, 4°C, for 10 minutes. The Sovall Legend RT tabletop refrigerated centrifuge is located in room 3957. Tubes should be placed in biosafety aerosol canister and each canister balanced prior to spinning.

10.2 With a marking pen, label two cryovials with the sequential collection ID numbers designated in the collection kit. The collection number suffix for serum is 04 and 05. For example, R160XXX 04, R160XXX 05.

10.3 Insert red colored cryocap inserts into the top of each cryovial used.

10.4 Place the corresponding cryovial label on the tube so that it does not cover up the marking pen labeling.

10.5 Carefully remove the cap from the Vacutainer tube and using a P-1000 pipet with a filter tip, transfer 1000ul of serum into pre labeled cryovial. Depending on the amount of serum in the tube, aliquots may be less than 1000ul.

10.6 Place the cap back on the Vacutainer tube and dispose of it and the pipet tip in the biohazard container located in the back of the laminar flow hood.

11. STORAGE

11.1 Filled cryovials may be temporary stored in the -20°C freezer until transfer to their permanent box in the -80°C freezer.

11.2 When time allows, place cryovials on dry ice and transfer to their permanent box in the -80°C.

11.3 Record the box and slot number of each vial on the collection event form.

SOP # **400-12** Subject **RNA Later Collection** Sheet **1** of **2**

Rev		Effective Date	
1/19/2019 Ver 4	**SOP for the UACC Biospecimen Repository**	10/18/16	Kepler

1. PURPOSE & SCOPE

The purpose of this standard operating procedure is to establish the collection of fresh tissue ~~for Dr. Benjamin Lee, Dr. Valentine Nfonsam and Dr. Steven Wang surgical cases.~~ Preservation in RNAlater when requested by an investigator.

2. REFERENCES

2.1 Collection Event Log
2.2 SOP# 400-10 Tissue Acquisition
2.3 RNAlater Handbook

3. RESPONSIBILITIES

3.1. Laboratory personnel collecting the specimens are responsible for following the procedures in this SOP and notifying the lab manager when deviations or unexpected events arise.
3.2. Laboratory personnel collecting the specimens are responsible for providing the filled out collection event log.
3.3. Laboratory personnel collecting the specimens are responsible for transferring the RNAlater vials containing tissue from 4C to -80C after 24 hours.

4. MATERIALS AND EQUIPMENT

4.1. Subject ID barcode affixed to a Collection Event form and Sample type log forms
4.2. Scalpels, forceps, Kimwipes, lead pencils and alcohol resistant marking.
4.3 RNAlater (Ambion)
4.4 Cryovials (USA Scientific)

5. PROCEDURES

5.1 Fill 1.5ml cryovials with 1ml of RNAlater. Vials can be filled several days in advance and stored on the collection Cart.

5.2 Follow the procedures outlined in SOP#400 Tissue Acquisition to obtain the tissue from pathology.

5.3 After tissue for banking is provided by pathology, Place the piece of tissue on a small section of modeling wax and lay that on the dissecting board. Using a sterile scalpel, cut the tissue into sections if it is large enough to divide it into three 5mm x 5mm size pieces. One piece should be frozen in OCT following the procedure in SOP#400-10, one piece in a cassette for FFPE preservation and the third piece into the RANlater containing vial and label the vial with the collection number, tissue type and date.

5.4 Return to the Biorepository lab and transfer the RNAlater tissue containing vial to the 4C refrigerator within the Biorepository lab.

5.5 After 24 hours, transfer the RNAlater tissue vial to the -80 freezer and record its location on the collection event log.

SOP # **400-13** Subject **Fresh Pancreatic Tissue Collection for the Jie/Heimark Laboratory** Sheet **1** of **2**

Rev		Effective Date	
01/24/2018 Ver 3	**SOP for the UACC Biospecimen Repository**	1/24/2018	Kepler

1. PURPOSE & SCOPE

The purpose of this standard operating procedure is to establish the collection of fresh pancreatic tumor in addition to the already established collections. See SOP# 400-10 for the University of Arizona Cancer Center Biorepository. The fresh specimens are to be a fee for service provided by TACMASR for Drs.Heimark and Merchant Labs.

2. DEFINITIONS

The Biospecimen Oversight Committee approved the acquisition of an additional portion of fresh tissue from Dr. Benjamin Lee's consented patients for the Kraft group research.

3. REFERENCES

SOP# 400-10
Collection Event Log
TACMASR billing worksheet
Biospecimen Oversight Committee approval form

RESPONSIBILITIES

3.1. Laboratory personnel collecting the specimens are responsible for following the procedures in this SOP and notifying the lab manager when deviations or unexpected events arise.

3.2. Laboratory personnel collecting the specimens are responsible for providing the filled out collection event log and TACMASR billing worksheet.

3.3. Laboratory personnel collecting the specimens are responsible for notifying Dr. Kraft's Lab for pickup of the fresh tissue.

4. MATERIALS AND EQUIPMENT

4.1. Subject ID barcode affixed to a Collection Event form and Sample type log forms
4.2. Scalpels, forceps, Kimwipes, lead pencils and alcohol resistant marking pen
4.3. Isopentane (2-methyl-butane) VWR JTQ223-8
4.4. Dewars for liquid nitrogen, and a small metal cup
4.5. Dry ice (bi-weekly from UA Cryogenics)
4.6. Ziploc bags (Bitran)
4.7. OCT Compound and cryomolds (Surgipath)
4.8. Vials containing 10% neutral buffered formalin (VWR)
4.9. 70% ethanol, diluted in DI water
4.10 Tissue cassettes (Surgipath)
4.11 500ml beaker full of Wet ice
4.12 biopsy punches
4.13 Media tube (provided day of collection by Heimark lab)

5. PROCEDURES

5.1 Follow the procedures for tissue procurement according to SOP# 400-10.
5.2 Dr. ~~Jie's~~ Heimark & Merchant labs are interested in collecting tissue from neuroendocrine tumor cases only.
5.2 On the day of surgery notify ~~Dr. Jie's post doc~~ Brenna (626-1912) and Suliman 535-1537 there will be fresh tissue available.
5.3 ~~Brenna~~ Both investigators should provide a tube of media on wet ice to TACMASR by 9am.
5.4 When paged go to the OR and retrieve the specimen. Take the specimen to pathology for grossing.
5.3 Pathology will ink and gross the pancreas.
5.4 From the tissue given to us, divide it into thirds, place one third into the media tube for ~~Dr. Jie's lab~~ Brenna, one third for Suliman and one third for TACMASR.
5.5 Label each tube with tumor, the TACMASR collection number and date.
5.6 Place the tube in a container of wet ice for transport to back to the biorepository lab.
5.7 Follow the procedure outlined in SOP# 400-10 for preserving tissue in OCT & FFPE with remaining specimen.
5.8 Fill out the TACMASR billing sheet with the Project name, date, and services provided.

SOP # **400-14** Subject **Fresh Lung Tissue Collection for the Martinez group** Sheet **1** of **2**

Rev		Effective Date	
09/08/17 Ver 2	**SOP for the UACC Biospecimen Repository**	6/19/14	McDaniel/ Kepler

1. PURPOSE & SCOPE

The purpose of this standard operating procedure is to establish the collection of fresh lung tumor and matching normal specimens in addition to the already established collections. See SOP# 400-10 for the University of Arizona Cancer Center Biorepository. The fresh specimens are to be a fee for service provided by TACMASR for Dr. Jesse Martinez's Lab.

2. DEFINITIONS

The Biospecimen Oversight Committee approved the acquisition of an additional portion of fresh tissue from Dr. Sam Kim's consented patients for the Martinez group research (June 2014)

3. REFERENCES

SOP# 400-10
Collection Event Log
TACMASR billing worksheet
Biospecimen Oversight Committee approval form

RESPONSIBILITIES

3.1. Laboratory personnel collecting the specimens are responsible for following the procedures in this SOP and notifying the lab manager when deviations or unexpected events arise.

3.2. Laboratory personnel collecting the specimens are responsible for providing the filled out collection event log and TACMASR billing worksheet.

3.3. Laboratory personnel collecting the specimens are responsible for notifying Dr. Martinez's Lab for delivery of the fresh tissue to Dr. Martinez's Lab.

4. MATERIALS AND EQUIPMENT

4.1. Subject ID barcode affixed to a Collection Event form and Sample type log forms
4.2. Scalpels, forceps, Kimwipes, lead pencils and alcohol resistant marking pen
4.3. Isopentane (2-methyl-butane) VWR JTQ223-8
4.4. Dewars for liquid nitrogen, and a small metal cup
4.5. Dry ice (bi-weekly from UA Cryogenics)
4.6. Ziploc bags (Bitran)
4.7. OCT Compound and cryomolds (Surgipath)
4.8. Vials containing 10% neutral buffered formalin (VWR)
4.9. 70% ethanol, diluted in DI water
4.10 Tissue cassettes (Surgipath)
4.11 500ml beaker full of Wet ice

5. PROCEDURES

5.1 **Follow** the procedures for tissue procurement according to SOP# 400-10.

5.2 In addition, on the day of surgery notify Dr. Martinez lab (Sara Centuori 626-2447) there will be fresh tissue available.
5.3 Prepare a 500ml beaker with wet ice.
5.4 When paged go to the OR and retrieve the specimen. Take the specimen to pathology for grossing.
5.3 From the portion of tumor provided to the Biorepository from the grossing pathology resident, cut off a small piece and place it in a pre-labeled 50 ml tube (Lung tumor and date), containing 10 ml of sterile PBS provided by Dr. Martinez Lab. The rest of the piece of tissue can be processed according to the Biorepository operating procedure SOP# 400-10.
5.4 Request a piece of matching normal from the grossing pathologist. Again, cut off a small piece of this and place in a separate, prelabeled 50ml tube (Lung, normal and date) containing 10ml of sterile PBS.
5.5 Place both 50ml tubes in the beaker of wet ice for transport to the Martinez Lab.
5.6 Fill out the TACMASR billing sheet with the Project name, date, and services provided.

SOP # **400-14** Subject **Fresh Lung Tissue Collection for the Martinez group** Sheet **1** of **2**

Rev		Effective Date	
09/08/17 Ver 2	**SOP for the UACC Biospecimen Repository**	6/19/14	McDaniel/ Kepler

1. PURPOSE & SCOPE

The purpose of this standard operating procedure is to establish the collection of fresh lung tumor and matching normal specimens in addition to the already established collections. See SOP# 400-10 for the University of Arizona Cancer Center Biorepository. The fresh specimens are to be a fee for service provided by TACMASR for Dr. Jesse Martinez's Lab.

2. DEFINITIONS

The Biospecimen Oversight Committee approved the acquisition of an additional portion of fresh tissue from Dr. Sam Kim's consented patients for the Martinez group research (June 2014)

3. REFERENCES

SOP# 400-10
Collection Event Log
TACMASR billing worksheet
Biospecimen Oversight Committee approval form

RESPONSIBILITIES

3.1. Laboratory personnel collecting the specimens are responsible for following the procedures in this SOP and notifying the lab manager when deviations or unexpected events arise.

3.2. Laboratory personnel collecting the specimens are responsible for providing the filled out collection event log and TACMASR billing worksheet.

3.3. Laboratory personnel collecting the specimens are responsible for notifying Dr. Martinez's Lab for delivery of the fresh tissue to Dr. Martinez's Lab.

4. MATERIALS AND EQUIPMENT

4.1. Subject ID barcode affixed to a Collection Event form and Sample type log forms
4.2. Scalpels, forceps, Kimwipes, lead pencils and alcohol resistant marking pen
4.3. Isopentane (2-methyl-butane) VWR JTQ223-8
4.4. Dewars for liquid nitrogen, and a small metal cup
4.5. Dry ice (bi-weekly from UA Cryogenics)
4.6. Ziploc bags (Bitran)
4.7. OCT Compound and cryomolds (Surgipath)
4.8. Vials containing 10% neutral buffered formalin (VWR)
4.9. 70% ethanol, diluted in DI water
4.10 Tissue cassettes (Surgipath)
4.11 500ml beaker full of Wet ice

5. PROCEDURES

5.1 **Follow** the procedures for tissue procurement according to SOP# 400-10.

5.2 In addition, on the day of surgery notify Dr. Martinez lab (Sara Centuori 626-2447) there will be fresh tissue available.
5.3 Prepare a 500ml beaker with wet ice.
5.4 When paged go to the OR and retrieve the specimen. Take the specimen to pathology for grossing.
5.3 From the portion of tumor provided to the Biorepository from the grossing pathology resident, cut off a small piece and place it in a pre-labeled 50 ml tube (Lung tumor and date), containing 10 ml of sterile PBS provided by Dr. Martinez Lab. The rest of the piece of tissue can be processed according to the Biorepository operating procedure SOP# 400-10.
5.4 Request a piece of matching normal from the grossing pathologist. Again, cut off a small piece of this and place in a separate, prelabeled 50ml tube (Lung, normal and date) containing 10ml of sterile PBS.
5.5 Place both 50ml tubes in the beaker of wet ice for transport to the Martinez Lab.
5.6 Fill out the TACMASR billing sheet with the Project name, date, and services provided.

SOP # **400-15** Subject **Fresh Prostate Tissue Collection for the Kraft Laboratory** Sheet **1** of **2**

Rev		Effective Date	
01/17/2018 Ver 1	**SOP for the UACC Biospecimen Repository**	1/17/2018	Kepler

1. PURPOSE & SCOPE

The purpose of this standard operating procedure is to establish the collection of fresh prostate tumor and matching normal specimens in addition to the already established collections. See SOP# 400-10 for the University of Arizona Cancer Center Biorepository. The fresh specimens are to be a fee for service provided by TACMASR for Dr. Andrew Kraft Lab.

2. DEFINITIONS

The Biospecimen Oversight Committee approved the acquisition of an additional portion of fresh tissue from Dr. Benjamin Lee's consented patients for the Kraft group research.

3. REFERENCES

SOP# 400-10
Collection Event Log
TACMASR billing worksheet
Biospecimen Oversight Committee approval form

RESPONSIBILITIES

3.1. Laboratory personnel collecting the specimens are responsible for following the procedures in this SOP and notifying the lab manager when deviations or unexpected events arise.

3.2. Laboratory personnel collecting the specimens are responsible for providing the filled out collection event log and TACMASR billing worksheet.

3.3. Laboratory personnel collecting the specimens are responsible for notifying Dr. Kraft's Lab for pickup of the fresh tissue.

4. MATERIALS AND EQUIPMENT

4.1. Subject ID barcode affixed to a Collection Event form and Sample type log forms
4.2. Scalpels, forceps, Kimwipes, lead pencils and alcohol resistant marking pen
4.3. Isopentane (2-methyl-butane) VWR JTQ223-8
4.4. Dewars for liquid nitrogen, and a small metal cup
4.5. Dry ice (bi-weekly from UA Cryogenics)
4.6. Ziploc bags (Bitran)
4.7. OCT Compound and cryomolds (Surgipath)
4.8. Vials containing 10% neutral buffered formalin (VWR)
4.9. 70% ethanol, diluted in DI water
4.10 Tissue cassettes (Surgipath)
4.11 500ml beaker full of Wet ice
4.12 Media tubes (provided day of collection by Kraft lab)
4.13 2mm punch biopsy

5. PROCEDURES

5.1 Follow the procedures for tissue procurement according to SOP# 400-10.
5.2 In addition, on the day of surgery notify Dr. Kraft's lab/Dr. Natalia Ignatenko (nai@email.arizona.edu) there will be fresh tissue available.
5.3 Dr. Ignatenko should provide two tubes with media on wet ice to TACMASR by 9am.
5.4 When paged go to the OR and retrieve the specimen. Take the specimen to pathology for grossing.
5.3 Pathology will ink and gross the prostate. Two punch biopsies of tumor are to be obtained and placed in the media containing tubes.
5.4 Two punch biopsies of normal are to be obtained and placed in a separate media containing tube.
5.5 Label each tube with tumor or normal, the TACMASR collection number and date.
5.6 Place both 50ml tubes in the beaker of wet ice for transport to back to the biorepository lab.
5.8 Notify Natalia that fresh tissue is available for pick up in TACMASR.
5.8 A separate punch biopsy of tumor and normal should also be collected for the Biorepository. Follow the procedure outlined in SOP# 400-10 for preserving tissue in OCT.
5.9 Fill out the TACMASR billing sheet with the Project name, date, and services provided.

SOP # **400-16** Subject **After Hours Tissue Collection** Sheet **1** of **2**

Rev		Effective Date	
07/24/2018 Ver 2		07/24/2018	Kepler

Materials

1. A liquid nitrogen dewer will be filled with LN2 by Tumor Bank Staff and left in ~~the frozen section room~~pathology on the ~~counter by the second dissecting hood~~. Table by the microscope.
2. A bag of cryovials will be stored beside the LN2 dewer
3. Marking pen will be stored with the cryovials
4. Collection Forms stored with the cryovials and LN2 dewer

Procedure

1. Tumor Bank staff will notify the on call resident of a possible collection.
2. The OR staff will be told by Tumor Bank staff to page pathology when the tissue is excised. Regardless of whether there is a frozen section or not. OR staff will be told pathology is collecting tissue and will need fresh tissue, no formalin added to it.
3. Tumor Bank Staff will update the pathology resident of on going cases.
4. Pathology resident will retrieve the tissue, gross it, and take a 0.5 x 0.5 cm sample of tumor (if possible) and normal tissue, (if possible). Each piece is to be put into a labeled cryovial with either tumor or normal, tissue type, date and patient last name.
5. Fill out the Collection form with the date, patient name and MRN, and the tissue collected.
6. Next drop the cryovails into the dewer of LN2.
7. Leave the LN2 dewer on the counter in the frozen section room and Tumor Bank staff will retrieve it the following morning.

Tumor Bank Contact

Carole Kepler B.S., MT(ASCP)
Lab phone: 626-6865
Cell phone: 520-747-0097
Pager: **8577**

~~Jocelyn Fimbres B.S.~~
~~Cell phone: 520-561-5571~~

TUMOR BANK Collection Form

Date: _____

Frozen By: _____

Patient Name: _____

Time: _____

Patient MRN: _____

Tumor Site: _____

Normal: _____

UNIVERSITY OF ARIZONA CANCER CENTER

NCI Comprehensive Cancer Center

A Cancer Center Designated by the National Cancer Institute

SOP # **400-17** Subject **Preparation of Urine Specimens** Sheet **1** of **3**

Rev		Effective Date	Author
1/19/2019 Ver 3	SOP for the UACC Biospecimen Repository	Sept 2018	Kepler

1. PURPOSE & SCOPE

To ensure proper collection, handling and preservation of urine specimens that are collected, processed and stored with in the Arizona Cancer Centers biorepository.

2. DEFINITIONS

TissueMetrix (AIM) is the biospecimen information management system administered through the University of Arizona Cancer Center.

3. REFERENCES

- Desktop SOP 400-5: Steps to Enroll Subjects and Bank Biospecimens
- Desktop SOP 400-9: Information Management
- Desktop SOP 400-11: Preparation of Blood Samples

4. RESPONSIBILITIES

4.1. TACMASR Laboratory research personnel who preparing samples for banking are responsible for following the procedures in the SOP and confirming that each step has been executed properly
4.2. Deviations are to be documented on the Collection Event Form
4.3. Unexpected events are to be reported to the Study Coordinator and/or Lab Director

5. SAFETY AND CAUTIONARY NOTES

5.1. Universal precautions and sterile technique should be followed. At minimum this should include the use of gloves, eye protection and lab coat. All processing of specimens should take place under a laminar flow hood.
5.2. Any spills or drops of urine should be immediately cleaned up by first spraying the spill area with 10% bleach, followed by 70% ethanol.
5.3. All spent containers, pipets and pipet tips are to be discarded in a red biohazard container.
5.4. If processing is to be delayed, store the urine specimen refrigerator in room 0915. If needed urine can be left overnight in the refrigerator and processed the following morning. This is not preferred however.
5.5. Complete the corresponding collection event form.

6. MATERIALS AND EQUIPMENT

6.1. 2.0ml Round Bottom, self-standing, cryogenic vials: VWR# 89094-810
6.2. Saf-T-Zip bag 6 x 9" Pack of 100 CAT# 11217-516 (Fisher).
6.3. 100ml sterile specimen container
6.4. 15ml sterile conical tubes VWR #79204-542
6.5. P-1000 Pipettors and sterile P-1000 filter pipette tips. USA Scientific #1122-1820
6.6. Sorvall Legend RT Tabletop refrigerated centrifuge located in Room 3957.
6.7 Biohazard container

7. URINE & BLOOD COLLECTION

7.1. Obtain the TissueMetrix Collection Event form and the sequential Specimen ID barcodes that are linked to the PTID. Refer to *SOP 400-9: Information Management* for details regarding the TissueMetrix labeling system.

~~7.2. The Biorepository requests two 6ml tubes of blood from consented study participants, K2 EDTA lavender top tube and a red top serum tube.~~

~~7.3.~~ Currently Urine is only collected from Dr. Chipollini's and Dr. Lee's patients.

7.4. ~~Pre-op is alerted by Biorepository personnel the evening before of the next day's consented patients~~.

7.5. After consenting the patient, place a Saf-T-zip bag with a specimen cup with the patients chart.

7.6. Urine will be collected in the OR.

7.7. The Biorepository is responsible for providing Pre-op a specimen cup and Saf-T-Zip bag to keep it in.

~~7.8.~~ When paged, Biorepository personnel proceeds to ~~Pre-op~~ the OR and retrieves the urine ~~& blood~~. ~~Pre-op puts the urine & blood in a basket labeled tumor bank on the main desk.~~

7.9. Record the patient information on the collection event form: name, MRN, DOS, DOB, sex, race/ethnicity, attending physician.

7.10. Record the time and date of urine & ~~blood~~ collection as well as the date and time of processing.

8. URINE PROCESSING

Refer to *SOP 400-9 Information Management,* for details regarding the TissueMetrix labeling system.

8.1. With a marking pen, label 4 cryovials with sequential collection ID numbers designated in the collection kit. The collection number suffix for urine is 08, 09, 10, and 11. For example R180XXX 08, R180XXX 09, R180XXX 10.

8.2. Place the corresponding cryolabel on the tube so that it does not cover up the marking pen labeling.

8.3. Carefully remove the cap from the urine containing specimen container and using a 10ml pipet, pipet 10ml's of urine into a sterile 15ml conical tube.

8.4. Dispose of 10ml pipet in the biohazard container.

8.5. Centrifuge the urine in the Sovall Legend RT tabletop refrigerated centrifuge located in room 0915. Tubes should be placed in biosafety aerosol canisters and each canister balanced prior to starting the centrifuge.

8.6. Centrifuge the urine at 1500rpm's for 5 minutes. Setting #4 on the centrifuge.

8.7. Using a P-1000 pipet with filter tip, transfer 1000ul of urine into each of the 4 labeled cryovials.

8.8. Place the lid back on the 15ml tube containing the remaining urine and dispose in the biohazard container.

8.9. The remainder of the urine in the specimen cup can also be disposed into the biohazard container.

9. STORAGE

11.1 Filled cryovials may be temporary stored in the -20°C freezer until transfer to their permanent box in the -80°C freezer.

11.2 When time allows, place cryovials on dry ice and transfer to their permanent box in the -80°C.

11.3 Record the box and slot number of each vial on the collection event form.

SOP # **400-18** Subject **Fresh Colon Tissue Collection for Dr. Ignateko** Sheet **1** of **2**

Rev		Effective Date	
01/192019 Ver 1	**SOP for the UACC Biospecimen Repository**	1/19/2019	Kepler

1. PURPOSE & SCOPE

The purpose of this standard operating procedure is to establish the collection of fresh colon tumor and matching normal specimens in addition to the already established collections. See SOP# 400-10 for the University of Arizona Cancer Center Biorepository. The fresh specimens are to be a fee for service provided by TACMASR for Dr. Natalia Ignateko.

2. DEFINITIONS

The Biospecimen Oversight Committee approved the acquisition of an additional portion of fresh tissue for Dr. Natalia Ignateko March 13, 2018.

3. REFERENCES

SOP# 400-10
Collection Event Log
TACMASR billing worksheet
Biospecimen Oversight Committee approval form

RESPONSIBILITIES

3.1. Laboratory personnel collecting the specimens are responsible for following the procedures in this SOP and notifying the lab manager when deviations or unexpected events arise.

3.2. Laboratory personnel collecting the specimens are responsible for providing the filled out collection event log and TACMASR billing worksheet.

3.3. Laboratory personnel collecting the specimens are responsible for notifying Dr. Ignateko for pickup of the fresh tissue.

4. MATERIALS AND EQUIPMENT

4.1. Subject ID barcode affixed to a Collection Event form and Sample type log forms
4.2. Scalpels, forceps, Kimwipes, lead pencils and alcohol resistant marking pen
4.3. Isopentane (2-methyl-butane) VWR JTQ223-8
4.4. Dewars for liquid nitrogen, and a small metal cup
4.5. Dry ice (bi-weekly from UA Cryogenics)
4.6. Ziploc bags (Bitran)
4.7. OCT Compound and cryomolds (Surgipath)
4.8. Vials containing 10% neutral buffered formalin (VWR)
4.9. 70% ethanol, diluted in DI water
4.10 Tissue cassettes (Surgipath)
4.11 500ml beaker full of Wet ice
4.12 Media tubes (provided day of collection by Dr. Ignateko)
4.13 2mm punch biopsy

5. PROCEDURES

5.1 Follow the procedures for tissue procurement according to SOP# 400-10.
5.2 In addition, on the day of surgery notify Dr. Natalia Ignatenko (nai@email.arizona.edu) there will be fresh tissue available.
5.3 Dr. Ignatenko should provide a tube with media on wet ice to TACMASR by 9am.
5.4 When paged go to the OR and retrieve the specimen. Take the specimen to pathology for grossing.
5.3 Pathology will ink and gross the colon. In addition to the piece of tumor collected for the biorepository, collect an additional piece and put it directly into the media tube supplied by Dr. Ignateko.
5.4 Label the tube with the TACMASR collection number and date.
5.6 Place the tube in the beaker of wet ice for transport to back to the biorepository lab.
5.8 Notify Dr. Ignateko that fresh tissue is available for pick up.
5.9 Fill out the TACMASR billing sheet with the Project name, date, and services provided.

Rev		Effective Date	
1/19/2019 Ver 5	**SOP for the UACC Biospecimen Repository**	February, 2012	Kepler

1. PURPOSE & SCOPE

To establish protocols within the UACC Biorepository to collect, process and transport from Banner University Medical Center to the Skin Institute laboratory in room 4975 within the University of Arizona Cancer Center. TACMASR is providing this as a fee for service for the Skin Institute.

2. DEFINITIONS

Skin Cancer Institute Patient Registry and Tissue Bank at the University of Arizona Cancer Center is directed by Drs. Clara Curiel, Robin Harris and Janine Einspahr, They have contracted with the TACMASR and the UACC Biorepository to consent, collect, process and deliver blood and tissue samples from pre-identified surgery cases. The Registry manages all data in the Skin Cancer silo of TissueMetrix.

Tissue Acquisition and Cellular/Molecular Analysis Shared Resource (TACMASR) is the core lab that is responsible for management of the UACC Biospecimen Repository (Tumor Bank). TACMASR also provides histological and pathological services to UACC investigators. http://azcc.arizona.edu/research/shared-services/tacmasr

TissueMetrix (AIM) is the biospecimen information management system administered through the University of Arizona Cancer Center.

3. REFERENCES

- SOPs from Registry
- SOP 400-5 Steps to Enroll Subjects and Bank Biospecimens
- SOP 400-10 Tissue Acquisition
- SOP 400-11 Blood processing and storage
- SOP 400-9 Information Management
- "Bloodborne Pathogen Exposure Control Plan." University of Arizona. Revised August 2003. Reference 29 CRF 1910.1030.
- TACMASR billing sheet
- SOP400-26 APPENDIX 1 *Key points to address when consenting participants.*

4. RESPONSIBILITIES

4.1. Biorepository and TACMASR personnel who are preparing samples for banking are responsible for following the procedures in the SOPs and confirming that each step has been properly executed

4.2. Deviations are to be documented on the Collection Event Form

4.3. Unexpected events are to be reported to the Coordinator, Lab Manager, and TACMASR Director

4.4. The Biospecimen Operations Manager and the TACMASR Lab Manager are responsible for training and daily oversight of personnel who perform tissue preparation procedures

5. SAFETY AND CAUTIONARY NOTES

5.1. Universal precautions and sterile technique should be followed. At minimum this should include the use of gloves, eye protection and lab coat.

5.2. All equipment and surfaces, forceps, dissecting board, that come in contact with tissue must be decontaminated with Converge upon the completion of tissue processing.
5.3. Used scalpels should be disposed of in the sharps container located on the transport cart.
5.4. Scrubs must be worn to enter the OR. Once in the frozen section room a disposable surgical cap, mask and shoe covers are available to don before entering the actual OR suite.
5.5. Acquisition of tissue is prioritized as follows:
5.5.1. Fresh frozen tissue, snap frozen in a cryovial dropped into liquid nitrogen
5.5.2. Formalin-fixed paraffin embedded (FFPE) tissue blocks.
5.6. Tissue can only be grossed and handed off to Biorepository personnel for banking by the pathology assistant, pathology resident or pathology attending.
5.7. Tissue should be snap frozen as soon as possible to preserve the morphology, protein biomarkers and RNA.
5.8. Prior arrangements are to be made with Mary Krutzsch in UACC Room 4975 to receive blood and frozen tissue.
5.9. Mary will also provide the Biorepository with a Skin Institute collection kit, that includes two labeled cryovials, one labeled tissue cassette, one specimen container with 10% buffered formalin and the Skin Institute collection log.

6. MATERIALS AND EQUIPMENT

6.1 Transport cart – refer to SOP 400-10 Appendix II for collection cart supplies
6.2 Skin Institute collection kit
6.3 Dry ice in a Styrofoam box
6.4 Dewer flask filled with liquid nitrogen

7. COLLECTION PROCEDURE

7.1 The Skin Institute Study coordinator identifies and notifies TACMASR with the patient name, MRN, DOS and surgeon for potential cases to be collected for them.
7.2 Biorepository personnel will monitor EPIC for the time of surgery and consent patient upon arrival.
7.3 Prepare the transport cart. Fill a Styrofoam box with dry ice, located in the cryobox in room 3959. Fill the liquid nitrogen dewar with liquid nitrogen, located in room 3959.
7.4 Verify the transport cart contains the rest of the items needed for collection. See SOP 400-10 Appendix II.
7.5 When the patient arrives in the pre-op facility meet them there for consenting. Biorepository personnel listed on the VOTF as consenters may consent.
7.6 Once the patient is consented, pre-op is notified that the patient is consented and blood is to be collected. The Skin Institute requests one purple EDTA tube and one red top serum tube.
7.7 When paged for blood collection, retrieve it and deliver it to Mary Krutzsch in UACC room 4975.
7.8 Monitor when the surgery has started from the OR status board within CERNER. Once the surgery has started telephone into the appropriate OR room to inform the circulating nurse the patient is consented for tissue collection. When calling in, state your name, that you are from Tumor Bank, the physician's name, and the patient's name. Give the OR nurse the Biorepository pager number, 8577
7.9 When the OR pages for tissue pickup, note the time of page on the skin collection form. Call the OR to verify that you received their page, the tissue has been excised and you will be in to pick it up.
7.10 Proceed with the transport cart to the frozen section room. Once inside there, prepare to enter the OR by donning protective apparel in the following order: surgical cap, surgical mask and shoe covers. Enter the OR suite and go directly to the specified OR room.
7.11 Cautiously enter the operating room. Do not touch anything sterile. Stand well back from the operation in progress, as close to the perimeter wall as possible. Locate the circulating nurse. He or she will hand over the tissue in a biospecimen container. Verify the label on the container is the correct patient and specimen.

7.12 Exit the OR suite and return to the frozen section room. Record the time of tissue collection on the skin collection form.

7.13 Place the specimen cup containing the tissue in the bottom drawer of the collection cart. Proceed to the Pathology will determine if there is enough tumor for banking. The size of the specimen provided to the biorepository depends on the amount and specimens banked. The Skin Institute makes the fresh snap frozen tissue the first priority and then if there is enough, a FFPE block.

7.14 Lay the piece of tissue received from pathology on a small sheet of dental wax. This is then placed on the dissecting board.

7.15 Using a sterile scalpel, cut the piece of tissue into two sections, if large enough. If not, freeze the entire specimen.

Procedure to snap freeze tissue

·Place the piece of tissue to be frozen in the pre-labeled cryovial in the skin collection kit.
·Fill the metal dewar bowl half way with liquid nitrogen.
·Drop the cryovial into the liquid nitrogen and leave there for 30 seconds.
·Using forceps, remove the vial and place in the Styrofoam box containing dry ice on the transport cart.
·Record the time frozen on the Skin collection form.
·Dispose of the scalpel in the biohazard container on the transport cart.
·Pour the remaining liquid nitrogen back into the liquid nitrogen dewar.
·Spray the forceps and dissecting board with Converge spray located in pathology.

Procedure to fix tissue in formalin for paraffin embedding

·Place the piece of tissue in the cassette provided in the skin collection kit and lock the lid in place.
·Drop the cassette into the specimen vial containing 10% neutral buffered formalin provided in the skin collection kit.
·Place a pink buffered formalin sticker on the side of the specimen cup. These are located in pathology by the formalin container.
·Note the time placed in formalin on the skin collection kit.
·Specimens should be fixed in formalin at room temperature between 5-24 hours. Do not leave in formalin over 24 hours, after this time the tissue is overfixed and the DNA will begin to fragment.

7.16 Deliver the frozen tissue along with the collection form to Mary Krutzsch in room 4975 of the UACC.

7.17 The FFPE sample remains with TACMASR to be processed.

7.18 Fill out a TACMASR billing form with the amount of time that was required for consenting, blood collection and delivery, and tissue collection and delivery.

APPENDIX 1

Key Points to address when consenting a potential participant:

-Banner University Medical Center is a research and teaching hospital affiliated with the Arizona Cancer Center.

-The Arizona Cancer Center is an NCI designated Comprehensive Cancer Center, 1 of 41 in the country.

-A requirement of being an NCI designated Comprehensive Cancer Center is to have a biorepository, in other words a big tissue bank.

-Because the surgeons at Banner University Medical Center participant in the tissue banking process, we are asking that a small piece of tissue that is removed during surgery be banked for future research. No additional tissue will be removed from you other than what is being removed as your standard of care. The tissue that pathology (the department of physicians that diagnosis your condition) doesn't need would be deemed extra tissue and is generally discarded. We ask if a small piece of that tissue could be put into the tissue bank.

-Tissue that goes into this bank can be used by researches here at the University of Arizona Cancer Center.

-This tissue is de-identified; a number is put on it so that any researcher using your tissue will not know your name.

-Study personnel at the Skin Institute will look at your medical records to get your age, Ethnicity and the pathology results (what your final diagnosis is).

-We will also take a small syringe of blood, this is done in pre-op when your IV is started, and thus no additional needle stick will be required.

-You do not benefit from being in the study

-There is NO cost to you for being in the study

-Frequently participants question agreeing to follow up check mark box (#4 on the Informed Consent). Stress they have the right to refuse this if they choose or to be removed from participating at any time.

-After signing, make them a copy of the signed consent.

Rev		Effective Date	Author
07/26/20 Ver 1	SOP for the BioDRoid Biospecimen Repository	Jan 2020	Chakrabarti/Zavros

1. PURPOSE & SCOPE

To ensure proper collection, handling and preservation of stomach tissy samples which are entered into the UACC Biology Development and Research of organoids (BioDRoid).

2. DEFINITIONS

TissueMetrix (AIM) is the biospecimen information management system administered through the University of Arizona Cancer Center.

3. REFERENCES

- Steps to Enroll Subjects and Bank Biospecimens
- Information Management

4. RESPONSIBILITIES

4.1. BioDRoid Laboratory research personnel who preparing samples for banking are responsible for following the procedures in the SOP and confirming that each step has been executed properly
4.2. Deviations are to be documented on the Collection Event Form
4.3. Unexpected events are to be reported to the Lab Manager or Lab Director

5. SAFETY AND CAUTIONARY NOTES

5.1. Universal precautions and sterile technique should be followed. At minimum this should include the use of gloves, eye protection and lab coat. All processing of specimens should take place under a laminar flow hood.
5.2. Any spills or drops of blood should be immediately cleaned up by first spraying the spill area with 10% bleach, followed by 70% ethanol.
5.3. All spent tubes and pipet tips are to be discarded in a red biohazard container.
5.4. Blood should be processed immediately.
5.5. If processing is to be delayed, store blood in the specimen refrigerator in room 0936. If needed blood can be stored overnight in the refrigerator and processed the following morning. This is not preferred however.
5.6. Complete the corresponding collection event form.

6. MATERIALS AND EQUIPMENT

6.1. K2 EDTA Vacutainer tubes, lavender top, plastic, 6 mls, BD367815 (VWR).
6.2. Serum Vacutainer tubes, red top, plastic, 6 ml tubes: BD367863 (VWR).
6.3. 2.0ml Round Bottom, self-standing, cryogenic vials # 03-337-7D (Fisher).
6.4. P-1000 Pipettors and sterile P-1000 filter pipette tips.
6.5. Sorvall Legend RT Tabletop refrigerated centrifuge located in Room 0936.
6.6. Lymphoprep # 7851 (STEMCELL Technologies)
6.7. RPMI # 10-040-CV (Thermo Fisher Scientific)
6.8. SepMateTM 50-IVD tube # 85450 (STEMCELL technologies)
6.9. Ficoll-Paque™ density gradient medium # 171440-02 (GE Healthcare)
6.10. AIMV Medium # 12055091 (Thermo Fisher Scientific)
6.11. Human serum AB # 21985023 (HSA, Gemini Bio-Products)
6.12. β-mercaptoethanol # 800-120 (Thermo Fisher Scientific)

6.13. Insulin-Transferrin-Selenium # 41400045 (ITS, Thermo fisher scientific)
6.14. Interleukin 2 # RP-8608 (IL-2, Thermo fisher scientific)
6.15. Interleukin 7 # RP-8645 (IL-7, Thermo fisher scientific)
6.16. Transforming growth factor beta 1 # 7754-BH-005/CF (TGF-β1 Thermo Fisher Scientific)
6.17. Vascular endothelial growth factor # RVEGFI (VEGF Thermo Fisher Scientific)
6.18. Prostaglandin E2 # P0409 (Sigma Aldrich)
6.19. Granulocyte-macrophage colony-stimulating factor # PHC6025 (GM-CSF, Thermo Fisher Scientific)
6.20. Interleukin 4 # RIL4I (IL-4, Thermo Fisher Scientific)
6.21. Tumor necrosis factor α # PHC3015 (TNF-α, Thermo Fisher Scientific)
6.22. Interleukin 1β # RIL1BI (IL-1β, Thermo Fisher Scientific)
6.23. Interleukin 6 # RIL6I (IL-6, Thermo Fisher Scientific)
6.24. EasySep buffer # 20144 (Stem Cell Technologies)
6.25. EasySep™ Human CD8+ T Cell Enrichment Kit # 19053 (Stemcell Technologies)
6.26. DMSO # D4540 (Sigma Aldrich)
6.27. Human serum AB # 21985023 (HSA, Gemini Bio-Products)
6.28. Biohazard containers

7. BLOOD COLLECTION

7.1. Obtain the TissueMetrix Collection Event form and the sequential Specimen ID barcodes that are linked to the PTID. Refer to *SOP : Information Management* for details regarding the TissueMetrix labeling system.
7.2. The Biorepository requests two 6ml tubes of blood from consented study participants, K2 EDTA lavender top tube and a red top serum tube.
7.3. Pre-op is alerted by Biorepository personnel the evening before of the next day's consented patients.
7.4. The Biorepository is responsible for stocking Pre-op with blood collection kits. Each kit (biospecimen bag) includes one red top tube, one lavender top tube and a Tissue Bank index card with the Biorepository phone number (626-7319) on it.
7.5. When called, Biorepository personnel proceeds to Pre-op and retrieve the blood. Pre-op puts the blood in a basket labeled tumor bank on the main desk.
7.6. Record the patient information on the collection event form: name, MRN, DOS, DOB, sex, race/ethnicity, attending physician.
7.7. Record the time and date of blood collection as well as the date and time of processing.

8. PLASMA PROCESSING

8.1 Centrifuge the remaining blood in the EDTA tube at 3000 RPM, 4°C, for 15 minutes. The Sovall Legend RT table top refrigerated centrifuge is located in room 0936.
8.2 With a marking pen, label two cryovials with sequential collection ID numbers designated in the collection kit. The collection number suffix for EDTA plasma is 01, and 02. For example, R160XXX 01, R160XXX 02.
8.3 Carefully remove the cap from the Vacutainer tube and using a P-1000 pipet with a filter tip, transfer 1000ul of plasma into each pre labeled cryovial. Depending on the amount of plasma in the tube, aliquots may be less than 1000ul.
8.4 Place the cap back on the Vacutainer tube and store the tube on ice for further processing of PBMCs.

9. SERUM PROCESSING

9.1 After red top Vacutainer has been allowed to clot for 30 minutes, centrifuge it at 3000 RPM, 4°C, for 15 minutes. The Sovall Legend RT tabletop refrigerated centrifuge is located in room 3957. Tubes should be placed in biosafety aerosol canister and each canister balanced prior to spinning.
9.2 With a marking pen, label two cryovials with the sequential collection ID numbers designated in the collection kit. The collection number suffix for serum is 04 and 05. For example, R160XXX 04, R160XXX 05.
9.3 Carefully remove the cap from the Vacutainer tube and using a P-1000 pipet with a filter tip, transfer 1000ul of serum into pre labeled cryovial. Depending on the amount of serum in the tube, aliquots may be less than 1000ul.
9.4 Place the cap back on the Vacut4ainer tube and dispose of it and the pipet tip in the biohazard container.

10. STORAGE

10.1 Filled cryovials be transfered to their permanent box in the -80°C freezer.
10.2 Record the box and slot number of each vial on the collection event form.

11. PBMC Processing using Ficoll-Paque™ density gradient medium

11.1 Dilute the blood from step 9.4 with an equal volume of DPBS.
11.2 Add 3-5 ml of Ficoll-Paque™ density gradient medium into a 15 ml Falcon® tube.
11.3 The recommended ratio is 3ml of Ficoll-Paque™ to 4 ml of the diluted blood sample.
11.4 Carefully layer the diluted blood sample onto the Ficoll-Paque™.
11.5 Centrifuge at 400 × g for an hour without brake.
11.6 Carefully transfer the mononuclear cells at the interface (buffy coat) into a new 15 ml Falcon® tube.
11.7 Dilute the collected mononuclear cells with 3 volumes of DPBS.
11.8 Centrifuge at 400 × g for 10 minutes. Discard supernatant. Repeat the centrifugation once more.
11.9 Resuspend pelleted cells in an appropriate culture medium for downstream applications or cryopreserve as frozen stocks.

12. PBMC Processing using Ficoll-Paque™ density gradient medium

12.1 Dilute the blood from step 9.4 with an equal volume of DPBS, supplemented with 1% fetal calf serum and 1% penicillin/streptomycin.
12.2 Add 15mL of LymphoprepTM to SepMateTM 50mL tube directly through the central hole.
12.3 Add the diluted blood slowly to the side of the SepMate tube, avoiding the central hole.
12.4 Centrifuge at 1200 x g for 10 minutes at 4oC.
12.5 Carefully and quickly pour the top layer into a separate 50mL conical and dilute 50% v/v with blood wash.
12.6 Centrifuge the supernatant enriched for leukocytes at 300 x g for 8 minutes at 4oC.
12.7 Discard the supernatant and re-suspend the pellet in 5mL Blood wash. At this point count the total number of PBMCs.
12.8 Centrifuge the leukocyte-enriched solution at 120 x g for 10 minutes at 4oC in order or separate leukocytes from platelets.
12.9 Resuspend the pellet in freezing media or proceed to culture.

13. Freezing PBMCs

13.1 Mix equal volume of stock Human Serum Albumin (HSA, 25%) and RPMI-1640 medium.
13.2 Prepare 2x Freezing Medium enough to resuspend 500µl/ cryovial. To prepare 2X freezing Media, mix equal volume of 25% HSA stock with sterile RPMI-1640 medium. Add 500µl DMSO per 1 ml of HSA/RPMI mixture.

13.3 Re-suspend PBMCs in 500uL of the 12.5% HSA. solution and pipet up and down to mix. Transfer PBMCs into a 2ml cryopreservation tube.
13.4 Add 500uL12.5% HSA solution to the into the cryopreservation tube.
13.5 Gently swirl tube while adding 500uL of 2x Freezing medium.
13.6 Immediately place the tube on ice. Do not mix any further.
13.7 Place in –80°C freezer. Do not store at Liquid Nitrogen.

14. CULTURE OF DENDRITIC CELLS (DCS)

14.1 Resuspend the pelleted PBMCs in AIMV basal medium.
14.2 Plate them in a 24 well tissue culture plate for 2 hours at 37°C in 5% CO2.
Composition of AIMV Basal Medium: AIM V medium containing 10% human serum AB, 1% Penicillin/Streptomycin and 50 µM β-mercaptoethanol.
14.3 Gently tap the culture plate and discard the culture medium containing non-adherent cells.
14.4 Slowly add AIMV DC culture medium to the adherent cells through the side of the plate.
14.5 Maintain cultures for 3 days at 37oC in 5% CO2. Replace with fresh culture medium on alternate days.
Composition of DC Culture Media: AIMV medium supplemented with 10% human serum AB, 1% Penicillin/Streptomycin, 50 µM β-mercaptoethanol, 800 U/ml granulocyte-macrophage colony-stimulating factor (GM-CSF) and 500 U/ml interleukin 4 (IL-4).
14.6 On day 3, discard the culture media and add fresh DC maturation medium.
14.7 Continue culturing the DCs for 24 hours at 37°C in 5% CO2.
Composition of DC Maturation Medium: DC culture medium additionally supplemented with 1% Penicillin/Streptomycin, 5 ng/ml tumor necrosis factor α (TNF-α), 5 ng/ml interleukin 1β (IL-1β), 150 ng/ml interleukin 6 (IL-6) and 1 µg/ml prostaglandin E2 (PGE2).

15. CULTURE OF CYTOTOXIC T-LYMPHOCYTES (CTLs)

15.1 Quickly thaw 1 vial of frozen PBMCs at 370C leaving one ice crystal. Add 1ml warm cRPMI slowly dropwise (over 30 seconds).
Composition of cRPMI: RPMI medium supplemented with 10% FCS and 1 % Penicillin/Streptomycin.
15.2 Transfer cells to a 15 ml conical tube.
15.3 Top up the tube with 3 ml warm cRPMI to cells and centrifuge at 300 x g for 5 minutes.
15.4 Resuspend PBMCs in 1ml EasySep buffer and transfer 1 ml of PBMCs to a 5 ml polystyrene round-bottom tube.
15.5 Add 50 µl of Enrichment Cocktail to the PBMCs. Incubate for 10 minutes at room temperature.
15.6 Add 150 µl of Magnetic Particles to the sample. Incubate for 5 minutes at room temperature.
15.7 Top up to 2.5 ml with EasySep™ Buffer. Place the polystyrene round-bottom tube into a magnet for 5 minutes to allow cell separation.
15.8 Pour off enriched cell suspension into a new 15 ml Falcon® tube and centrifuge at 300 × g for 5 minutes.
15.9 Discard the supernatant and resuspend the cells in CTL culture medium.
15.10 Seed the cells in a 24 well cell culture plate and continue culturing CTLs for 24 hours at 37° C in 5% CO2.
Composition of CTL Culture Media: RPMI 1640 medium containing 10% human serum AB, 1% Penicillin/Streptomycin, 50 µM β-mercaptoethanol, 1 × Insulin-Transferrin-Selenium, 0.15 µg/ml interleukin 2 (IL-2) and 0.1 µg/ml interleukin 7 (IL-7)2 hours at 37°C in 5% CO2.

16. CULTURE OF DENDRITIC CELLS (DCS)

16.1 Quickly thaw 1 vial of frozen PBMCs at 370C leaving one ice crystal. Add 1ml warm cRPMI slowly dropwise (over 30 seconds).
Composition of cRPMI: RPMI medium supplemented with 10% FCS and 1 % Penicillin/Streptomycin.

16.2 Transfer cells to a 15 ml conical tube.
16.3 Top up the tube with 3 ml warm cRPMI to cells and centrifuge at 300 x g for 5 minutes.
16.4 Resuspend PBMCs in 1ml EasySep buffer and transfer 1 ml of PBMCs to a 5 ml polystyrene round-bottom tube.
16.5 Add 50 µl of Enrichment Cocktail to the PBMCs. Incubate for 10 minutes at room temperature.
16.6 Add 150 µl of Magnetic Particles to the sample. Incubate for 5 minutes at room temperature.
16.7 Top up to 2.5 ml with EasySep™ Buffer. Place the polystyrene round-bottom tube into a magnet for 5 minutes to allow cell separation.
16.8 Pour off enriched cell suspension into a new 15 ml Falcon® tube and centrifuge at 300 × g for 5 minutes.
16.9 Discard the supernatant and resuspend the cells in CTL culture medium.
16.10 Seed the cells in a 24 well cell culture plate and continue culturing CTLs for 24 hours at 37o C in 5% CO2.
Composition of CTL Culture Media: RPMI 1640 medium containing 10% human serum AB, 1% Penicillin/Streptomycin, 50 µM β-mercaptoethanol, 1 × Insulin-Transferrin-Selenium, 0.15 µg/ml interleukin 2 (IL-2) and 0.1 µg/ml interleukin 7 (IL-7).

17. CULTURE OF MYELOID DERIVED SUPPRESSOR CELLS (MDSCs)

17.1 Culture PBMCs in AIM V MDSC culture medium for 7 days at 37oC in 5% CO2 to enrich for MDSCs.
17.2 Replace with fresh culture medium every alternate day and continue culturing MDSCs until used in organoid – immune cell co-culture.
Composition of MDSC Culture Media: AIMV medium containing 50% conditioned medium collected from organoid cultures, and supplemented with 1% Penicillin/Streptomycin, 10 ng/ml IL-1β, 10 ng/ml IL-6, 1 µg/ml PGE2, 2 ng/ml transforming growth factor beta 1 (TGF-β1), 10 ng/ml TNF-α, 10 ng/ml vascular endothelial growth factor (VEGF) and 10 ng/ml GM-CSF.

18. ORGANOID IMMUNE CELL CO-CULTURE

Procedure: DCs and CTL Co-Culture

18.1 Collect conditioned medium from organoid cultures. The media should be conditioned by organoids for 7 days. Conditioned media can be stored at -20°C until used for cultures (avoid freeze thawing).
18.2 Gently remove 50% of the culture media from the DCs and replace with the organoid conditioned media.
18.3 Incubate DCs with conditioned media for 2h at 37°C in 5% CO2.
18.4 Collect loosely adherent DCs with a pipette and centrifuge at 300X g for 5min at 4°C.
18.5 Remove supernatant and save pellet on ice to resuspend with CTLs (next step).
18.6 Harvest the CTLs and transfer them to the matured DCs. Continue DC-CTL co-culture for 72 hours at 37oC in 5% CO2.
Composition of RPMI Co-Culture Media: RPMI 1640 medium supplemented with 10% human serum AB and 1% Penicillin/Streptomycin.

Procedure: Organoid- Immune Cell Co-culture

18.7 Isolate the CTLs from DC-CTL co-cultures using the EasySep™ Human CD8+T Cell Enrichment Kit.
18.8 Incubate CTLs with 1mL 5µM carboxyfluorescein diacetate succinimidyl ester (CFSE) at 37°C for 20min.
18.9 Quench the staining by adding five times DPBS-FCS medium. Centrifuge the CTLs at 300X g for 5min at 4°C.
Composition of DPBS-FCS media: DPBS supplemented with 10% fetal calf serum and 1% penicillin/streptomycin.
18.10 Resuspend the CTLs in 1mL 3D gastric organoid medium and incubate for a further 10min at 37°C.
18.11 Harvest organoids in cold DPBS and centrifuge at 400 g for 5min at 4°C.
18.12 Mix the organoids with CFSE-labeled CTLs. Resuspend in an appropriate volume of thawed Matrigel® on ice. The volumes are determined based on the experimental design required for the studies.

18.13 When MDSCs are required within the co-culture, a 1:4 (CTL:MDSC) and 1:1 (CTL:organoids) is used for co-culture.

18.14 Seed as 30–50µL Matrigel® droplets containing organoids plus CTLs in 48 or 24 well tissue culture plates.

18.15 Incubate plates at 37°C for 15min to allow the cell-Matrigel® droplets to be solidified.

18.16 Overlay cell-Matrigel® droplets with pre-warmed 3D gastric organoid culture medium. When seeding into 48 or 24 well plates use 250 µL or 500 µL of medium respectively.

18.17 Maintain organoid/immune cell co-cultures at 37°C in 5% CO2 for 24h.

18.18 After 24h, treat the co-culture with appropriate drugs or inhibitors and maintain the culture at 37°C in 5% CO_2 for 48–72h based on experimental needs.
NOTE: CFSE is a blue laser dye that can be used for analyzing CTL proliferation using flow cytometry. For Experimental conditions requiring MDSCs, mix MDSCs together with the organoids and CFSE-labeled CTLs before seeding them in the culture plates.

SOP # Subject **Preparation of Colon Organoids** Sheet **1** of **3**

Rev		Effective Date	Author
07/26/20 Ver 1	SOP for the BioDRoid Biospecimen Repository	Jan 2020	Chakrabarti/Zavros

1. PURPOSE & SCOPE

To ensure proper collection, handling and preservation of stomach tissue samples which are entered into the UACC Biology Development and Research of organoids (BioDRoid).

2. DEFINITIONS

TissueMetrix (AIM) is the biospecimen information management system administered through the University of Arizona Cancer Center.

3. REFERENCES

- Steps to Enroll Subjects and Bank Biospecimens
- Information Management

4. RESPONSIBILITIES

4.1. BioDRoid Laboratory research personnel who preparing samples for banking are responsible for following the procedures in the SOP and confirming that each step has been executed properly
4.2. Deviations are to be documented on the Collection Event Form
4.3. Unexpected events are to be reported to the Lab Manager or Lab Director

5. SAFETY AND CAUTIONARY NOTES

5.1. Universal precautions and sterile technique should be followed. At minimum this should include the use of gloves, eye protection and lab coat. All processing of specimens should take place under a laminar flow hood.
5.2. Any spills or drops of blood should be immediately cleaned up by first spraying the spill area with 10% bleach, followed by 70% ethanol.
5.3. All spent tubes and pipet tips are to be discarded in a red biohazard container.
5.4. Blood should be processed immediately.
5.5. If processing is to be delayed, store blood in the specimen refrigerator in room 0936. If needed blood can be stored overnight in the refrigerator and processed the following morning. This is not preferred however.
5.6. Complete the corresponding collection event form.

6. MATERIALS AND EQUIPMENT

6.1. Advanced Dulbecco's modified Eagle medium/F12 medium (Thermo Fisher Scientific, 12634010).
6.2. GlutaMAX # 350-50-061 (Fisher Scientific)
6.3. Penicillin/Streptomycin # SV30010 (Thermo Fisher Scientific)
6.4. Amphotericin B / Gentamicin # R-01510 (Thermo Fisher Scientific)
6.5. Kanamycin Sulfate # 11815024 (Thermo Fisher Scientific)
6.6. HEPES Buffer # BP299-100 (Fisher Scientific)
6.7. n-Acteylcystine # A7250 (Sigma Aldrich)
6.8. N2 # 17502048 (Thermo Fisher Scientific)
6.9. B27 # 12587010 (Thermo Fisher Scientific)
6.10. Epidermal Growth Factor # 236-EG-01M (EGF, R&D Systems)
6.11. Nicotinamide # N0636 (Sigma Aldrich)
6.12. Y-27632 ROCK inhibitor # 125410 (Tocris)

6.13. SB202190 # 12-641-0 (Tocris)
6.14. Fetal bovine serum # SI2450H (FBS, Atlanta Biologicals)
6.15. Matrigel™ # CB40230C (Corning)
6.16. Ca2+/Mg2+ -free Dulbecco's Phosphate Buffered Saline # 14190-144 (DPBS, Fisher Scientific)
6.17. Collagenase Type 3 # 150704 (MP Biomedicals)
6.18. 5ml Round bottom polystyrene tubes # 14956-3C (Fisher Scientific)
6.19. TZV (THIAZOVIVIN) # SML1045 (SIGMA)
6.20. CHIR99021 # 4423 (Tocris)
6.21. A8301 # A2939 (Tocris)
6.22. T-175 Tissue culture flask # 431038 (Fisher Corning)
6.23. 0.25% Trypsin/EDTA # 25200056 (Thermo Fisher)
6.24. Freezing media # 12648-010 (Thermofisher)
6.25. 150 mm Tissue culture Petri dish # 430599 (Fisher)
6.26. Bottle-Top Filters with 0.22μm Membrane # 430513 (Fisher)
6.27. 500 mL Sterile Collection bottles # 430282 (Fisher Sci)
6.28. L-WRN cells # L-3276 (ATCC)
6.29. G418 # 15710064 (Thermofisher Scientific)
6.30. Hygromycin # 10687010 (Thermofisher Scientific)
6.31. Biohazard containers

7. TISSUE COLLECTION

7.1. Obtain the TissueMetrix Collection Event form and the sequential Specimen ID barcodes that are linked to the PTID. Refer to *SOP : Information Management* for details regarding the TissueMetrix labeling system.
7.2. The Biorepository requests normal or tumor biopsies/resected tissues from consented study participants, in 50ml tube containing collection media.
7.3. Pre-op is alerted by Biorepository personnel the evening before of the next day's consented patients.
7.4. The Biorepository is responsible for stocking Pre-op with blood collection kits. Each kit (biospecimen bag) includes two 50ml tube, 10ml collection media and a Tissue Bank index card with the Biorepository phone number (626-7319) on it.
(Collection Media contains, Advanced DMEM/F12 media supplemented with 2 mM GlutaMAX, 1% Penicillin/Streptomycin, 10 mM HEPES Buffer, 0.25 mg/mL Amphotericin B /10 mg/mL Gentamicin, 1% Kanamycin, 1x N2, 1x B27, 1 mM N-acetyl-L-cysteine, 10nM Nicotinamide, 100nM A8301, 4μM CHIR, and 2.5 μM Thiazovivin).
7.5. When called, Biorepository personnel proceeds to Pre-op and retrieve the tissue. Pre-op puts the tissue on a ice bucket labeled tumor bank on the main desk.
7.6. Record the patient information on the collection event form: name, MRN, DOS, DOB, sex, race/ethnicity, attending physician.
7.7. Record the time and date of tissue collection as well as the date and time of processing.

8. CRYOPRESERRATTION OF TISSUE

8.1 Wash Tissue with DPBS + Antibiotics (DPBS supplemented with 1% Penicillin/Streptomycin, 0.25 mg/mL Amphotericin B /10 mg/mL Gentamicin, 1% Kanamycin)
8.2 Cut the tissue into smaller pieces with razor blades in a petri dish
8.3 Transfer tissue to a cryovial with freezing media

(Freezing media composition: 70% colon organoid growth media, supplemented with 20% FBS, 10% DMSO, 100nM A8301, 4μM CHIR, and 2.5 μM Thiazovivin)

8.4 Store overnight at -80°C inside Mr. Frosty containing 250mL Isopropanol, 24hrs

8.5 Transfer the vial to liquid nitrogen for long term storage

8.6 Dispose all waste and the pipet tip in the biohazard container.

9. THAWING OF CRYOPRESERVED TISSUE

9.1 Thaw tissue at 37°C water bath, leaving one ice crystal left

9.2 Slowly add 1ml pre-warmed thawing media dropwise to the vial
(Thawing media composition: colon organoid growth media, supplemented with 100nM A8301, 4μM CHIR, and 2.5 μM Thiazovivin).

9.3 Remove the media

9.4 Place tissue in a petri dish containing thawing media

9.5 Mince to smaller pieces with razor blades in a petri dish, proceed to digestion

9.6 Dispose all waste and the pipet tip in the biohazard container.

10. GENERATION OF HUMAN-DERIVED TUMOR COLON ORGANOIDS

10.1 Mince the tissues using surgical scalpel blades on a cell culture Petri dish. Wash the fragmented tissues with 5-10 ml of DPBS supplemented with antibiotics.

10.2 Transfer the tissue into a 15 ml Falcon® tube.

10.3 Add 5-10 ml of pre-warmed digestion buffer to the tissues, depending on the tissue size.
(Composition of digestion buffer: DPBS supplemented with 1mg/mL Collagenase Type 3).

10.4 Incubate the tissue at 37°C for 15-30 minutes in an orbital shaker, depending on the size and consistency of the tissues. Check for the appearance of cell clusters under the microscope, every 10 minutes.

10.5 Dilute the digestion buffer by adding twofold DPBS-antibiotics.

10.6 Centrifuge at 400 × g for 5 minutes at 4°C to pellet cells.

10.7 Discard the supernatant and resuspend the pellet with cold DPBS plus antibiotics.

10.8 Centrifuge at 400 × g for 5 minutes at 4°C.

10.9 Carefully remove the supernatant and store the cells on ice.

10.10 Resuspend cell pellet in an appropriate volume of Matrigel® Seed 50 μl cell-Matrigel® droplets in 24 – 12 well cell culture treated plates.

10.11 Incubate the cell-Matrigel™ droplets at 37°C for 15 minutes to solidify as a dome.

10.12 Add 500 ml-1 ml pre-warmed 3D colon organoid culture medium to overlay cell-Matrigel™ dome.
(Composition of 3D Colon Organoid Culture Media: 50% of 2x Basal media (Advanced Dulbecco's modified Eagle medium/F12 medium supplemented with 4mM L-glutamine, 1% Penicillin/Streptomycin, 20 mM HEPES (2-[4-(2-hydroxyethyl) piperazin-1-yl] ethanesulfonic acid), 2 mM N-acetyl-L-cysteine (Sigma-Aldrich), 2× N-2 supplement, 2x B27 supplement), 50% LWRN-conditioned medium, 100 μg/mL Epidermal Growth Factor (EGF), 10 nM Nicotinamide, and 10 μM Y-27632 ROCK inhibitor, 500nM A83-01, 10μM SB 202190, 2.5μM CHIR99021).

10.13 Maintain the 3D organoid cultures at 37°C in 5% CO_2. Replace with 50% fresh medium every 1-2 days depending on the organoid growth.

10.14 Passage organoids once every 7-10 days in 1:3 ratio, based on the organoid density.

10.15 Dispose all waste and the pipet tip in the biohazard container.

11. PASSAGING AND EXPANSION OF ORGANOIDS

11.1 Carefully aspirate media from each well

11.2 Add 1mL cold DPBS (-Ca & -Mg)/well

11.3 Harvest organoids and transfer them to round bottom 5mL tube (3wells/tube)
11.4 Centrifuge @ 400 x g, 5 min
11.5 Carefully remove the supernatant.
11.6 Add 1mL prewarmed TrypLE and 10mM Y-27632/ tube
11.7 Incubate @ 37°C, 6 min
11.8 Pipette up and down for 10 times in medium speed.
11.9 Check under a microscope for small clusters
11.10 Add 4ml DPBS/tube
11.11 Centrifuge @ 400 x g, 5 min
11.12 Carefully remove the supernatant.
11.13 Resuspend the pellet with the desired amount of Matrigel™
11.14 Carefully add 50μL Matrigel™ per well of a 12 well tissue culture plate, 30μL per well of an 8 well chamber slide, 40μL per well of a 24 well chamber slide, 20μL per well of a 48 well chamber slide
11.15 Add pre warmed growth media in each well
11.16 Change media every 3-4 days and continue culture with organoid growth media.
11.17 Dispose all waste and the pipet tip in the biohazard container.

12. CRYOPRESERVATION OF ORGANOIDS

12.1 Carefully aspirate media from each well
12.2 Add 1mL cold DPBS (-Ca & -Mg)/well
12.3 Harvest organoids and transfer them to round bottom 5mL tube (3wells/tube)
12.4 Centrifuge @ 400 x g, 5 min
12.5 Carefully remove the supernatant.
12.6 Add 1mL prewarmed TrypLE and 10mM Y-27632/ tube
12.7 Incubate @ 37°C, 6 min
12.8 Syringe 5 times with 26g needle (medium speed)
12.9 Check under a microscope for small clusters
12.10 Add 4ml cold DPBS/tube
12.11 Centrifuge @ 400 x g, 5 min
12.12 Carefully remove the supernatant.
12.13 Resuspend the pellet with the freezing media (1ml/vial) (Section 8.3).
12.14 Store overnight at -80°C inside Mr. Frosty containing 250mL Isopropanol, 24hrs
12.15 Transfer the vial to liquid nitrogen for long term storage
12.16 Dispose all waste and the pipet tip in the biohazard container.

13. THAWING AND CULTURING OF CRYOPRESERVED ORGANOIDS

13.1 Quickly thaw 1vial of frozen vial of organoid in 37°C water bath, leaving a small crystal of ice.
13.2 Slowly add 1mL pre warmed thawing media (Section 9.2) dropwise to each cryovial
13.3 Transfer the cells to a 5mL round bottom tube
13.4 Add 1mL thawing media
13.5 Centrifuge @ 400 x g, 5 min
13.6 Carefully remove the supernatant.
13.7 Resuspend the pellet with required volume of cold matrigel.
13.8 Carefully add matrigel bubble to each plate
13.9 Incubate @ 37°C, 13 min
13.10 Add pre warmed thawing media in each well.

13.11 After 48hrs, remove media and continue culture with organoid growth media.
13.12 Dispose all waste and the pipet tip in the biohazard container.

14. CULTURE OF LWRN CONDITIONED MEDIA PRODUCING CELLS

14.1 Quickly thaw 1vial of frozen vial of organoid in 37°C water bath, leaving a small crystal of ice.
14.2 Transfer the thawed cells to a 15 mL tube and add 9 mL growth media.
14.3 Centrifuge at 500xg, 5 min
14.4 Remove supernatant
14.5 Plate L-WRN cells in to a 150mm cell culture petridish, containing 30mL complete growth media (500mL Advanced DMEM/F12 + 20% FCS+ 1% Penicillin/Streptamycin).
14.6 The next day, depending on cell recovery, change media with dual selection (0.5mg/ml G418 and 0.5 mg/ml Hygromycin) and grow to about 80-90% confluency (usually ~ five days) without media change.
14.7 Passage cells into 10 new plates without antibiotic selection.
14.8 Remove media and wash with 10 mL warm DPBS, without Ca/Mg
14.9 Add 10 mL 0.25% trypsin/EDTA, incubate at 37°C, 5 min
14.10 Collect cells in to a 50mL tube
14.11 Centrifuge at 500xg, 5 min
14.12 Resuspend pellet in 10 mL media (1ml cell/ dish)
14.13 Add 25 mL of Growth Media per dish.
14.14 Once cells are about 80% confluent (usually ~2days), collect the conditioned media and replace with fresh media on each plate every day for 12 consecutive days.
14.15 Save 500 µL of media into a centrifuge tube from each day for testing activity using the TOPflash Wnt Reporter testing. Store at 4°C.
14.16 Centrifuge the conditioned media every day at 500xg for 10 minutes, to allow debris to settle at the bottom.
14.17 Filter sterilize the conditioned media every day and store the media in 500mL sterile bottle.
14.18 Combine conditioned media from day 1-4, 5-8 and 9-12.
14.19 Aliquot into 25- or 50-mL volumes and store aliquots at -80^{o} C.
14.20 Dispose all waste and the pipet tip in the biohazard container.

Rev		Effective Date	Author
07/26/20 Ver 1	SOP for the BioDRoid Biospecimen Repository	Jan 2020	Chakrabarti/Zavros

1. PURPOSE & SCOPE

To ensure proper collection, handling and preservation of stomach tissue samples which are entered into the UACC Biology Development and Research of organoids (BioDRoid).

2. DEFINITIONS

TissueMetrix (AIM) is the biospecimen information management system administered through the University of Arizona Cancer Center.

3. REFERENCES

- Steps to Enroll Subjects and Bank Biospecimens
- Information Management

4. RESPONSIBILITIES

4.1. BioDRoid Laboratory research personnel who preparing samples for banking are responsible for following the procedures in the SOP and confirming that each step has been executed properly
4.2. Deviations are to be documented on the Collection Event Form
4.3. Unexpected events are to be reported to the Lab Manager or Lab Director

5. SAFETY AND CAUTIONARY NOTES

5.1. Universal precautions and sterile technique should be followed. At minimum this should include the use of gloves, eye protection and lab coat. All processing of specimens should take place under a laminar flow hood.
5.2. Any spills or drops of blood should be immediately cleaned up by first spraying the spill area with 10% bleach, followed by 70% ethanol.
5.3. All spent tubes and pipet tips are to be discarded in a red biohazard container.
5.4. Blood should be processed immediately.
5.5. If processing is to be delayed, store blood in the specimen refrigerator in room 0936. If needed blood can be stored overnight in the refrigerator and processed the following morning. This is not preferred however.
5.6. Complete the corresponding collection event form.

6. MATERIALS AND EQUIPMENT

6.1. Advanced Dulbecco's modified Eagle medium/F12 medium (Thermo Fisher Scientific, 12634010).
6.2. GlutaMAX # 350-50-061 (Fisher Scientific)
6.3. Penicillin/Streptomycin # SV30010 (Thermo Fisher Scientific)
6.4. Amphotericin B / Gentamicin # R-01510 (Thermo Fisher Scientific)
6.5. Kanamycin Sulfate # 11815024 (Thermo Fisher Scientific)
6.6. HEPES Buffer # BP299-100 (Fisher Scientific)
6.7. n-Acteylcystine # A7250 (Sigma Aldrich)
6.8. N2 # 17502048 (Thermo Fisher Scientific)
6.9. B27 # 12587010 (Thermo Fisher Scientific)
6.10. Bone morphogenetic protein inhibitor # 250-38 (Noggin, Peprotech)
6.11. Gastrin # 30061 (Tocris Biosciences)
6.12. Epidermal Growth Factor # 315-09 (EGF, Peprotech)

6.13. Fibroblast growth factor 10 # 100-26 (FGF10, Peprotech)
6.14. Nicotinamide # N0636 (Sigma Aldrich)
6.15. Y-27632 ROCK inhibitor # Y0503 (Sigma Aldrich)
6.16. L cells, a Wnt3a producing cell line (Hubrecht Institute for Developmental Biology and Stem Cell Research, Netherlands).
6.17. Modified HEK-293T R-spondin secreting cell line
6.18. Dulbecco's Modified Eagle Medium (DMEM) # 12634-010 (Thermo Fisher Scientific)
6.19. Fetal bovine serum # SI2450H (FBS, Atlanta Biologicals)
6.20. OPTIMEM # 51985-034(Thermo Fisher Scientific)
6.21. Zeocin # R25001 (Thermo Fisher)
6.22. Matrigel™ # CB40230C (Corning)
6.23. Ca2+/Mg2+ -free Dulbecco's Phosphate Buffered Saline # 14190-144 (DPBS, Fisher Scientific)
6.24. EDTA # E6758 (Sigma Aldrich)
6.25. Hyaluronidase Type IV-S # H3884 (Sigma-Aldrich).
6.26. Collagenase Type 1A # C9891 (Sigma-Aldrich)
6.27. BSA # A7906 (Sigma Aldrich)
6.28. 5ml Round bottom polystyrene tubes # 14956-3C (Fisher Scientific)
6.29. HBSS # 14175095 (Thermo Fisher Scientific)
6.30. 40micron filter # 352340 (Fisher Scientific)
6.31. TZV (THIAZOVIVIN) # SML1045 (SIGMA)
6.32. CHIR99021 # SML1046 (SIGMA)
6.33. A8301 # A2939 (Tocris)
6.34. DMEM+ Glutamax-1 # 10569-010 (Thermo Fisher)
6.35. Optimem + Glutamax 1 # 51985-034 (Thermo Fisher)
6.36. Zeocin (Thermo Fisher # R25001)
6.37. T-175 Tissue culture flask # 431038 (Fisher Corning)
6.38. 0.25% Trypsin/EDTA # 25200056 (Thermo Fisher)
6.39. Freezing media # 12648-010 (Thermofisher)
6.40. 150 mm Tissue culture Petri dish # 430599 (Fisher)
6.41. Bottle-Top Filters with 0.22µm Membrane # 430513 (Fisher)
6.42. 500 mL Sterile Collection bottles # 430282 (Fisher Sci)
6.43. Biohazard containers

7. TISSUE COLLECTION

7.1. Obtain the TissueMetrix Collection Event form and the sequential Specimen ID barcodes that are linked to the PTID. Refer to *SOP : Information Management* for details regarding the TissueMetrix labeling system.
7.2. The Biorepository requests normal or tumor biopsies/resected tissues from consented study participants, in 50ml tube containing collection media.
7.3. Pre-op is alerted by Biorepository personnel the evening before of the next day's consented patients.
7.4. The Biorepository is responsible for stocking Pre-op with blood collection kits. Each kit (biospecimen bag) includes two 50ml tube, 10ml collection media and a Tissue Bank index card with the Biorepository phone number (626-7319) on it.
(Collection Media contains, Advanced DMEM/F12 media supplemented with 2 mM GlutaMAX, 1% Penicillin/Streptomycin, 10 mM HEPES Buffer, 0.25 mg/mL Amphotericin B /10 mg/mL Gentamicin, 1%

Kanamycin, 1x N2, 1x B27, 1 mM N-acetyl-L-cysteine, 10mM Nicoinamide, 100nM A8301, 4μM CHIR, and 2.5 μM Thiazovivin).

7.5. When called, Biorepository personnel proceeds to Pre-op and retrieve the tissue. Pre-op puts the tissue on a ice bucket labeled tumor bank on the main desk.

7.6. Record the patient information on the collection event form: name, MRN, DOS, DOB, sex, race/ethnicity, attending physician.

7.7. Record the time and date of tissue collection as well as the date and time of processing.

8. CRYOPRESERRATTION OF TISSUE

8.1 Wash Tissue with DPBS + Antibiotics (DPBS supplemented with 1% Penicillin/Streptomycin, 0.25 mg/mL Amphotericin B /10 mg/mL Gentamicin, 1% Kanamycin)

8.2 Cut the tissue into smaller pieces with razor blades in a petri dish

8.3 Transfer tissue to a cryovial with freezing media
(Freezing media composition: 70% gastric organoid growth media, supplemented with 20% FBS, 10% DMSO, 100nM A8301, 4μM CHIR, and 2.5 μM Thiazovivin)

8.4 Store overnight at -80°C inside Mr. Frosty containing 250mL Isopropanol, 24hrs

8.5 Transfer the vial to liquid nitrogen for long term storage

8.6 Dispose all waste and the pipet tip in the biohazard container.

9. THAWING OF CRYOPRESERVED TISSUE

9.1 Thaw tissue at 37°C water bath, leaving one ice crystal left

9.2 Slowly add 1ml pre-warmed thawing media dropwise to the vial
(Thawing media composition: gastric organoid growth media, supplemented with 100nM A8301, 4μM CHIR, and 2.5 μM Thiazovivin).

9.3 Remove the media

9.4 Place tissue in a petri dish containing thawing media

9.5 Mince to smaller pieces with razor blades in a petri dish, and proceed to digestion

9.6 Dispose all waste and the pipet tip in the biohazard container.

10. GENERATION OF HUMAN-DERIVED TUMOR GASTRIC ORGANOIDS

10.1 Mince the tissues using surgical scalpel blades on a cell culture Petri dish. Wash the fragmented tissues with 5-10 ml of DPBS supplemented with antibiotics.

10.2 Transfer the tissue into a 50 ml Falcon® tube. Add 5-10 ml of pre-warmed EDTA stripping buffer, depending on the size of the minced tissues.
(Composition of EDTA stripping buffer: Hank's Balanced Salt Solution (HBSS) supplemented with 5 mM EDTA, 25 mM HEPES, and 10% heat FCS).

10.3 Incubate the tissue at 37°C for 10 minutes. Carefully remove the EDTA buffer and add fresh 10 ml EDTA buffer.

10.4 Continue incubation at 37°C for another 5 minutes.

10.5 Take off the EDTA buffer, wash twice with 10 mL DMEM-antibiotics (no centrifugation needed).

10.6 Add 5-10 ml of pre-warmed digestion buffer to the tissues, depending on the tissue size.
(Composition of digestion buffer: Gastric organoid culture media supplemented with 1.5 mg/mL Collagenase Type 1A and, 0.4 mg/mL Hyaluronidase Type IV-S).

10.7 Incubate the tissue at 37°C for 15-30 minutes in an orbital shaker, depending on the size and consistency of the tissues. Check for the appearance of cell clusters under the microscope, every 10 minutes.

10.8 Dilute the digestion buffer by adding twofold cold DMEM-antibiotics.

10.9 Filter undigested tissues through 40micron filters and collect the flow through.
10.10 Centrifuge the flow through at 400 × g for 5 minutes at 4oC to pellet cells.
10.11 Discard the supernatant and resuspend the pellet with cold DPBS plus antibiotics.
10.12 Centrifuge at 400 × g for 5 minutes at 4°C.
10.13 Carefully remove the supernatant and store the cells on ice.
10.14 Resuspend cell pellet in an appropriate volume of Matrigel® Seed 50 µl cell-Matrigel® droplets in 24 – 12 well cell culture treated plates.
10.15 Incubate the cell-Matrigel™ droplets at 37°C for 15 minutes to solidify as a dome.
10.16 Add 500 ml-1 ml pre-warmed 3D gastric organoid culture medium to overlay cell-Matrigel™ dome.
(Composition of 3D Gastric Organoid Culture Media: Advanced Dulbecco's modified Eagle medium/F12 medium supplemented with 2mM L-glutamine, 1% Penicillin/Streptomycin, 10 mM HEPES (2-[4-(2-hydroxyethyl) piperazin-1-yl] ethanesulfonic acid), 1 mM N-acetyl-L-cysteine (Sigma-Aldrich), 1× N-2 supplement, 1 x B27 supplement, 50% Wnt3a-conditioned medium, 10% R-spondin-conditioned medium, 100 ng/mL bone morphogenetic protein inhibitor (Noggin), 1 nM gastrin 1, 50 ng/mL Epidermal Growth Factor (EGF), 200 ng/mL Fibroblast growth factor 10 (FGF10), 10 mM Nicotinamide, and 10 µM Y-27632 ROCK inhibitor).
10.17 Maintain the 3D organoid cultures at 37°C in 5% CO2. Replace with fresh medium every 3-4 days depending on the organoid growth.
10.18 Passage organoids once every 7-10 days in 1:2 or 1:3 ratio, based on the organoid density.
10.19 Dispose all waste and the pipet tip in the biohazard container.

11. PASSAGING AND EXPANSION OF ORGANOIDS

11.1 Carefully aspirate media from each well
11.2 Add 1mL cold DPBS (-Ca & -Mg)/well
11.3 Harvest organoids and transfer them to round bottom 5mL tube (3wells/tube)
11.4 Centrifuge @ 400 x g, 5 min
11.5 Carefully remove the supernatant.
11.6 Add 1mL prewarmed Accutase/ tube
11.7 Incubate @ 37°C, 6 min
11.8 Syringe 5 times with 26g needle (medium speed)
11.9 Check under a microscope for small clusters
11.10 Add 4ml cold DPBS/tube
11.11 Centrifuge @ 400 x g, 5 min
11.12 Carefully remove the supernatant.
11.13 Resuspend the pellet with the desired amount of Matrigel™
11.14 Carefully add 50µL Matrigel™ per well of a 12 well tissue culture plate, 30µL per well of an 8 well chamber slide, 40µL per well of a 24 well chamber slide, 20µL per well of a 48 well chamber slide
11.15 Add pre warmed growth media in each well
11.16 Change media every 3-4 days and continue culture with organoid growth media.
11.17 Dispose all waste and the pipet tip in the biohazard container.

12. CRYOPRESERVATION OF ORGANOIDS

12.1 Carefully aspirate media from each well
12.2 Add 1mL cold DPBS (-Ca & -Mg)/well
12.3 Harvest organoids and transfer them to round bottom 5mL tube (3wells/tube)
12.4 Centrifuge @ 400 x g, 5 min
12.5 Carefully remove the supernatant.

12.6 Add 1mL prewarmed Accutase/ tube
12.7 Incubate @ 37°C, 6 min
12.8 Syringe 5 times with 26g needle (medium speed)
12.9 Check under a microscope for small clusters
12.10 Add 4ml cold DPBS/tube
12.11 Centrifuge @ 400 x g, 5 min
12.12 Carefully remove the supernatant.
12.13 Resuspend the pellet with the freezing media (1ml/vial) (Section 8.3).
12.14 Store overnight at -80°C inside Mr. Frosty containing 250mL Isopropanol, 24hrs
12.15 Transfer the vial to liquid nitrogen for long term storage
12.16 Dispose all waste and the pipet tip in the biohazard container.

13. THAWING AND CULTURING OF CRYOPRESERVED ORGANOIDS

13.1 Quickly thaw 1vial of frozen vial of organoid in 37°C water bath, leaving a small crystal of ice.
13.2 Slowly add 1mL pre warmed thawing media (Section 9.2) dropwise to each cryovial
13.3 Transfer the cells to a 5mL round bottom tube
13.4 Add 1mL thawing media
13.5 Centrifuge @ 400 x g, 5 min
13.6 Carefully remove the supernatant.
13.7 Resuspend the pellet with required volume of cold matrigel.
13.8 Carefully add matrigel bubble to each plate
13.9 Incubate @ 37°C, 13 min
13.10 Add pre warmed thawing media in each well.
13.11 After 48hrs, remove media and continue culture with organoid growth media.
13.12 Dispose all waste and the pipet tip in the biohazard container.

14. CULTURE OF Wnt CONDITIONED MEDIA PRODUCING L CELLS

14.1 Quickly thaw 1vial of frozen vial of organoid in 37°C water bath, leaving a small crystal of ice.
14.2 Plate L cells in T-175 flasks, containing 40mL complete Media (500mL DMEM+ 10% FCS+ 1% Pen/Strep) plus 50uL Zeocin.
14.3 Let the cells grow up to 90% confluency (5-7 days)
14.4 Passage cells in to 7 x T175 culture flasks
14.5 Remove media and wash with 10 mL warm DPBS, without Ca/Mg
14.6 Add 10 mL 0.25% trypsin/EDTA, incubate at 37°C, 5 min
14.7 Collect cells in to a 50mL tube
14.8 Centrifuge at 800xg, 5 min
14.9 Resuspend pellet in 7 mL media
14.10 Add 1 mL cells/1 T175 containing 40 mL media
14.11 2xT175 will get zeocin rests are not
14.12 When 2, + zeocin T175 becomes 70% confluent, they can be passaged (1x T175 to 7x T175) or frozen down (1x T175 to 8 aliquots).
14.13 The other 5x T175s washed with 10 mL warm DPBS/flask, without Ca/Mg
14.14 Add 10 mL 0.25% trypsin/EDTA, incubate at 37°C / flask
14.15 Add 10 mL media / flask and collect all cells in to 50mL tubes
14.16 Centrifuge at 800xg, 5 min

14.17 Resuspend pellet in 10 mL media
14.18 Add 10 mL cells into 500mL complete media bottle
14.19 Add 23 mL cells in to each of 25 x 150mm petri dishes
14.20 Let them grow for 1 week
14.21 Collect Wnt condition media, filter, aliquot, label and store at -80°C
14.22 Dispose all waste and the pipet tip in the biohazard container.

15. CULTURE OF R spondin CONDITIONED MEDIA PRODUCING HEK293T CELLS

15.1 Quickly thaw 1vial of frozen vial of organoid in 37°C water bath, leaving a small crystal of ice.
15.2 Plate L cells in T-175 flasks, containing 40mL complete Media (500mL DMEM+ 10% FCS+ 1% Pen/Strep) plus 50uL Zeocin.
15.3 Let the cells grow up to 90% confluency (5-7 days)
15.4 Passage cells in to 7 x T175 culture flasks
15.5 Remove media and wash with 10 mL warm DPBS, without Ca/Mg
15.6 Add 10 mL 0.25% trypsin/EDTA, incubate at 37°C, 5 min
15.7 Collect cells in to a 50mL tube
15.8 Centrifuge at 800xg, 5 min
15.9 Resuspend pellet in 7 mL media
15.10 Add 1 mL cells/1 T175 containing 40 mL media
15.11 2xT175 will get zeocin rests are not
15.12 When 2, + zeocin T175 becomes 70% confluent, they can be passaged (1x T175 to 7x T175) or frozen down (1x T175 to 8 aliquots).
15.13 The other 5x T175s washed with 10 mL warm DPBS/flask, without Ca/Mg
15.14 Add 10 mL 0.25% trypsin/EDTA, incubate at 37°C / flask
15.15 Add 10 mL media / flask and collect all cells in to 50mL tubes
15.16 Centrifuge at 800xg, 5 min
15.17 Resuspend pellet in 10 mL media
15.18 Add 10 mL cells into 500mL complete media bottle
15.19 Add 23 mL cells in to each of 25 x 150mm petri dishes
15.20 After 24hrs, change media of all 25 dishes with 500 mL Optimem + 1% Pen/Step (no FCS), 20mL/dish
15.21 Let them grow for 1 week
15.22 Collect R spondin condition media, filter, aliquot, label and store at -80°C
15.23 Dispose all waste and the pipet tip in the biohazard container.

SOP # Subject **Preparation of Pancreatic Cancer Organoids** Sheet **1** of **3**

Rev		Effective Date	Author
07/26/20 Ver 1	SOP for the BioDRoid Biospecimen Repository	Jan 2020	Chakrabarti/Zavros

1. PURPOSE & SCOPE

To ensure proper collection, handling and preservation of stomach tissue samples which are entered into the UACC Biology Development and Research of organoids (BioDRoid).

2. DEFINITIONS

TissueMetrix (AIM) is the biospecimen information management system administered through the University of Arizona Cancer Center.

3. REFERENCES

- Steps to Enroll Subjects and Bank Biospecimens
- Information Management

4. RESPONSIBILITIES

4.1. BioDRoid Laboratory research personnel who preparing samples for banking are responsible for following the procedures in the SOP and confirming that each step has been executed properly
4.2. Deviations are to be documented on the Collection Event Form
4.3. Unexpected events are to be reported to the Lab Manager or Lab Director

5. SAFETY AND CAUTIONARY NOTES

5.1. Universal precautions and sterile technique should be followed. At minimum this should include the use of gloves, eye protection and lab coat. All processing of specimens should take place under a laminar flow hood.
5.2. Any spills or drops of blood should be immediately cleaned up by first spraying the spill area with 10% bleach, followed by 70% ethanol.
5.3. All spent tubes and pipet tips are to be discarded in a red biohazard container.
5.4. Blood should be processed immediately.
5.5. If processing is to be delayed, store blood in the specimen refrigerator in room 0936. If needed blood can be stored overnight in the refrigerator and processed the following morning. This is not preferred however.
5.6. Complete the corresponding collection event form.

6. MATERIALS AND EQUIPMENT

6.1. Advanced Dulbecco's modified Eagle medium/F12 medium (Thermo Fisher Scientific, 12634010).
6.2. GlutaMAX # 350-50-061 (Fisher Scientific)
6.3. Penicillin/Streptomycin # SV30010 (Thermo Fisher Scientific)
6.4. Amphotericin B / Gentamicin # R-01510 (Thermo Fisher Scientific)
6.5. B27 # 12587010 (Thermo Fisher Scientific)
6.6. Fibroblast growth factor 10 # 100-26 (FGF10, Peprotech)
6.7. Y-27632 ROCK inhibitor # Y0503 (Sigma Aldrich)
6.8. Ascorbic acid # 4055-50 (R&D Systems)
6.9. Insulin # 3435/10 (R&D Systems)
6.10. Hydrocortisone # H0888-1G (Sigma Aldrich)
6.11. Fibroblast growth factor-basic #100-18B (FGF2, Peprotech)

6.12. All trans Retinoic Acid # R2625 (ATRA, Sigma)
6.13. Bovine pituitary extract # P1476 (BPE, Sigma,),
6.14. L cells, a Wnt3a producing cell line (Hubrecht Institute for Developmental Biology and Stem Cell Research, Netherlands).
6.15. Modified HEK-293T R-spondin secreting cell line
6.16. Dulbecco's Modified Eagle Medium (DMEM) # 12634-010 (Thermo Fisher Scientific)
6.17. Fetal bovine serum # SI2450H (FBS, Atlanta Biologicals)
6.18. OPTIMEM # 51985-034(Thermo Fisher Scientific)
6.19. Zeocin # R25001 (Thermo Fisher)
6.20. Matrigel™ # CB40230C (Corning)
6.21. Ca2+/Mg2+ -free Dulbecco's Phosphate Buffered Saline # 14190-144 (DPBS, Fisher Scientific)
6.22. Collagenase P # 11 249 002 001 (Sigma-Aldrich)
6.23. 5ml Round bottom polystyrene tubes # 14956-3C (Fisher Scientific)
6.24. HBSS # 14175095 (Thermo Fisher Scientific)
6.25. 70micron filter # 22363548 (Fisher Scientific)
6.26. TZV (THIAZOVIVIN) # SML1045 (SIGMA)
6.27. CHIR99021 # SML1046 (SIGMA)
6.28. A8301 # A2939 (Tocris)
6.29. DMEM+ Glutamax-1 # 10569-010 (Thermo Fisher)
6.30. Optimem + Glutamax 1 # 51985-034 (Thermo Fisher)
6.31. Zeocin (Thermo Fisher # R25001)
6.32. T-175 Tissue culture flask # 431038 (Fisher Corning)
6.33. 0.25% Trypsin/EDTA # 25200056 (Thermo Fisher)
6.34. Freezing media # 12648-010 (Thermofisher)
6.35. 150 mm Tissue culture Petri dish # 430599 (Fisher)
6.36. Bottle-Top Filters with 0.22μm Membrane # 430513 (Fisher)
6.37. 500 mL Sterile Collection bottles # 430282 (Fisher Sci)
6.38. Biohazard containers

7. TISSUE COLLECTION

7.1. Obtain the TissueMetrix Collection Event form and the sequential Specimen ID barcodes that are linked to the PTID. Refer to *SOP : Information Management* for details regarding the TissueMetrix labeling system.
7.2. The Biorepository requests normal or tumor biopsies/resected tissues from consented study participants, in 50ml tube containing collection media.
7.3. Pre-op is alerted by Biorepository personnel the evening before of the next day's consented patients.
7.4. The Biorepository is responsible for stocking Pre-op with blood collection kits. Each kit (biospecimen bag) includes two 50ml tube, 10ml collection media and a Tissue Bank index card with the Biorepository phone number (626-7319) on it.
(Collection Media contains, Advanced DMEM/F12 media supplemented with 1% Penicillin/Streptomycin, 0.25 mg/mL Amphotericin B /10 mg/mL Gentamicin, 1% Kanamycin, 1x B27, 100nM A8301, 4μM CHIR, and 2.5 μM Thiazovivin).
7.5. When called, Biorepository personnel proceeds to Pre-op and retrieve the tissue. Pre-op puts the tissue on a ice bucket labeled tumor bank on the main desk.

7.6. Record the patient information on the collection event form: name, MRN, DOS, DOB, sex, race/ethnicity, attending physician.
7.7. Record the time and date of tissue collection as well as the date and time of processing.

8. CRYOPRESERRATTION OF TISSUE

8.1 Wash Tissue with DPBS + Antibiotics (DPBS supplemented with 1% Penicillin/Streptomycin, 0.25 mg/mL Amphotericin B /10 mg/mL Gentamicin, 1% Kanamycin)
8.2 Cut the tissue into smaller pieces with razor blades in a petri dish
8.3 Transfer tissue to a cryovial with freezing media
(Freezing media composition: 70% pancreatic organoid growth media, supplemented with 20% FBS, 10% DMSO, 100nM A8301, 4μM CHIR, and 2.5 μM Thiazovivin)
8.4 Store overnight at -80°C inside Mr. Frosty containing 250mL Isopropanol, 24hrs
8.5 Transfer the vial to liquid nitrogen for long term storage
8.6 Dispose all waste and the pipet tip in the biohazard container.

9. THAWING OF CRYOPRESERVED TISSUE

9.1 Thaw tissue at 37°C water bath, leaving one ice crystal left
9.2 Slowly add 1ml pre-warmed thawing media dropwise to the vial
(Thawing media composition: pancreatic organoid growth media, supplemented with 100nM A8301, 4μM CHIR, and 2.5 μM Thiazovivin).
9.3 Remove the media
9.4 Place tissue in a petri dish containing thawing media
9.5 Mince to smaller pieces with razor blades in a petri dish, and proceed to digestion
9.6 Dispose all waste and the pipet tip in the biohazard container.

10. GENERATION OF HUMAN TUMOR-DERIVED PANCREATIC ORGANOIDS

10.1 Mince the tissues using surgical scalpel blades on a cell culture Petri dish. Wash the fragmented tissues with 5-10 ml of DPBS supplemented with antibiotics.
10.2 Add tissue to 5mL pancreatic wash buffer with 5mg collagenase P (1mg collagenase P/mL)
(Composition of wash buffer: HBSS supplemented with 1% Penicillin/Streptomycin, 0.25 mg/mL Amphotericin B /10 mg/mL Gentamicin, 1% Kanamycin, 5% FCS).
10.3 Vortex and Incubate in 37°C shaker for 15-45 mins
10.4 Check for small clusters of cells under a microscope every 15 min.
10.5 Add 5ml pancreatic wash buffer to tissue. (1:1 v/v)
10.6 Run through 70-micron sterile filter.
10.7 Rinse filter with pancreatic wash buffer.
10.8 Centrifuge at 400g for 5mins
10.9 Resuspend pellet in PBS+ Antibiotic and transfer cells to a 5mL round bottom tube
10.10 Centrifuge @ 40 x g, 5min
10.11 Carefully remove the supernatant.
10.12 Resuspend the pellet with the desired amount of Matrigel™
10.13 Plate 50μL Matrigel™ per well of a 12 well tissue culture plate
10.14 Add pre-warmed thawing media in each well

(Composition of pancreatic growth media: advanced DMEM/F12, 1X B27 , 284 μM ascorbic acid, 20 μg/μL Insulin, 0.25 μg/μL hydrocortisone, 100 ng/mL fibroblast growth factor-basic (FGF2), 100 nM all trans Retinoic Acid (ATRA), 10 μM Y27632, 100 ng/mL fibroblast growth factor 10 (FGF10), 1% Penicillin/Streptomycin, 0.1% Gentamicin/ Amphotericin B, 2 mM glutamax and 56 μg/mL bovine pituitary extract, 10% R-Spondin, and 50% Wnt conditioned media)

10.15 Maintain the 3D organoid cultures at 37°C in 5% CO2. Replace with fresh medium every 3-4 days depending on the organoid growth.

10.16 Passage organoids once every 7-10 days in 1:2 or 1:3 ratio, based on the organoid density.

10.17 Dispose all waste and the pipet tip in the biohazard container.

11. PASSAGING AND EXPANSION OF ORGANOIDS

11.1 Carefully aspirate media from each well

11.2 Add 1mL cold DPBS (-Ca & -Mg)/well

11.3 Harvest organoids and transfer them to round bottom 5mL tube (3wells/tube)

11.4 Centrifuge @ 400 x g, 5 min

11.5 Carefully remove the supernatant.

11.6 Add 1mL prewarmed Accutase/ tube

11.7 Incubate @ 37°C, 6 min

11.8 Syringe 5 times with 26g needle (medium speed)

11.9 Check under a microscope for small clusters

11.10 Add 4ml cold DPBS/tube

11.11 Centrifuge @ 400 x g, 5 min

11.12 Carefully remove the supernatant.

11.13 Resuspend the pellet with the desired amount of Matrigel™

11.14 Carefully add 50μL Matrigel™ per well of a 12 well tissue culture plate, 30μL per well of an 8 well chamber slide, 40μL per well of a 24 well chamber slide, 20μL per well of a 48 well chamber slide

11.15 Add pre warmed growth media in each well

11.16 Change media every 3-4 days and continue culture with organoid growth media. (Pancreatic growth media contains

11.17 Dispose all waste and the pipet tip in the biohazard container.

12. CRYOPRESERVATION OF ORGANOIDS

12.1 Carefully aspirate media from each well

12.2 Add 1mL cold DPBS (-Ca & -Mg)/well

12.3 Harvest organoids and transfer them to round bottom 5mL tube (3wells/tube)

12.4 Centrifuge @ 400 x g, 5 min

12.5 Carefully remove the supernatant.

12.6 Add 1mL prewarmed Accutase/ tube

12.7 Incubate @ 37°C, 6 min

12.8 Syringe 5 times with 26g needle (medium speed)

12.9 Check under a microscope for small clusters

12.10 Add 4ml cold DPBS/tube

12.11 Centrifuge @ 400 x g, 5 min

12.12 Carefully remove the supernatant.

12.13 Resuspend the pellet with the freezing media (1ml/vial) (Section 8.3).

12.14 Store overnight at -80°C inside Mr. Frosty containing 250mL Isopropanol, 24hrs
12.15 Transfer the vial to liquid nitrogen for long term storage
12.16 Dispose all waste and the pipet tip in the biohazard container.

13. THAWING AND CULTURING OF CRYOPRESERVED ORGANOIDS

13.1 Quickly thaw 1vial of frozen vial of organoid in 37°C water bath, leaving a small crystal of ice.
13.2 Slowly add 1mL pre warmed thawing media (Section 9.2) dropwise to each cryovial
13.3 Transfer the cells to a 5mL round bottom tube
13.4 Add 1mL thawing media
13.5 Centrifuge @ 400 x g, 5 min
13.6 Carefully remove the supernatant.
13.7 Resuspend the pellet with required volume of cold matrigel.
13.8 Carefully add matrigel bubble to each plate
13.9 Incubate @ 37°C, 13 min
13.10 Add pre warmed thawing media in each well.
13.11 After 48hrs, remove media and continue culture with organoid growth media.
13.12 Dispose all waste and the pipet tip in the biohazard container.

14. CULTURE OF Wnt CONDITIONED MEDIA PRODUCING L CELLS

14.1 Quickly thaw 1vial of frozen vial of organoid in 37°C water bath, leaving a small crystal of ice.
14.2 Plate L cells in T-175 flasks, containing 40mL complete Media (500mL DMEM+ 10% FCS+ 1% Pen/Strep) plus 50uL Zeocin.
14.3 Let the cells grow up to 90% confluency (5-7 days)
14.4 Passage cells in to 7 x T175 culture flasks
14.5 Remove media and wash with 10 mL warm DPBS, without Ca/Mg
14.6 Add 10 mL 0.25% trypsin/EDTA, incubate at 37°C, 5 min
14.7 Collect cells in to a 50mL tube
14.8 Centrifuge at 800xg, 5 min
14.9 Resuspend pellet in 7 mL media
14.10 Add 1 mL cells/1 T175 containing 40 mL media
14.11 2xT175 will get zeocin rests are not
14.12 When 2, + zeocin T175 becomes 70% confluent, they can be passaged (1x T175 to 7x T175) or frozen down (1x T175 to 8 aliquots).
14.13 The other 5x T175s washed with 10 mL warm DPBS/flask, without Ca/Mg
14.14 Add 10 mL 0.25% trypsin/EDTA, incubate at 37°C / flask
14.15 Add 10 mL media / flask and collect all cells in to 50mL tubes
14.16 Centrifuge at 800xg, 5 min
14.17 Resuspend pellet in 10 mL media
14.18 Add 10 mL cells into 500mL complete media bottle
14.19 Add 23 mL cells in to each of 25 x 150mm petri dishes
14.20 Let them grow for 1 week
14.21 Collect Wnt condition media, filter, aliquot, label and store at -80°C
14.22 Dispose all waste and the pipet tip in the biohazard container.

15. CULTURE OF Rspondin CONDITIONED MEDIA PRODUCING HEK293T CELLS

15.1 Quickly thaw 1vial of frozen vial of organoid in 37°C water bath, leaving a small crystal of ice.
15.2 Plate L cells in T-175 flasks, containing 40mL complete Media (500mL DMEM+ 10% FCS+ 1% Pen/Strep) plus 50uL Zeocin.
15.3 Let the cells grow up to 90% confluency (5-7 days)
15.4 Passage cells in to 7 x T175 culture flasks
15.5 Remove media and wash with 10 mL warm DPBS, without Ca/Mg
15.6 Add 10 mL 0.25% trypsin/EDTA, incubate at 37°C, 5 min
15.7 Collect cells in to a 50mL tube
15.8 Centrifuge at 800xg, 5 min
15.9 Resuspend pellet in 7 mL media
15.10 Add 1 mL cells/1 T175 containing 40 mL media
15.11 2xT175 will get zeocin rests are not
15.12 When 2, + zeocin T175 becomes 70% confluent, they can be passaged (1x T175 to 7x T175) or frozen down (1x T175 to 8 aliquots).
15.13 The other 5x T175s washed with 10 mL warm DPBS/flask, without Ca/Mg
15.14 Add 10 mL 0.25% trypsin/EDTA, incubate at 37°C / flask
15.15 Add 10 mL media / flask and collect all cells in to 50mL tubes
15.16 Centrifuge at 800xg, 5 min
15.17 Resuspend pellet in 10 mL media
15.18 Add 10 mL cells into 500mL complete media bottle
15.19 Add 23 mL cells in to each of 25 x 150mm petri dishes
15.20 After 24hrs, change media of all 25 dishes with 500 mL Optimem + 1% Pen/Step (no FCS), 23mL/dish
15.21 Let them grow for 1 week
15.22 Collect Rspondin condition media, filter, aliquot, label and store at -80°C
15.23 Dispose all waste and the pipet tip in the biohazard container.

UACC Biorepository

Standard Operating Procedures

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University of Arizona Cancer Center
Room 0917 Phone (520) 626-7319
UACC-TACMASS@uacc.arizona.edu

TACMASR OPERATING PROCEDURE

SOP #0 | Subject **Leica Embedder Cleaning & Maintenance** | Sheet **1** of **1**

Rev 0		Effective Date	Author
10/27/2017		20/27/2017	Fimbres

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