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We use this protocol and it's working

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WUSTL Senescence Network (SenNet) Biospecimen Collection SOP

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DISCLAIMER

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ABSTRACT

This protocol describes the tissue collection and storing aspect of Washington University's Biospecimen core as part of the Cellular Senescence Network (SenNet).

IMAGE ATTRIBUTION

Images created by Matthew Wyczalkowski

MATERIALS

Reagents:

1. Dry Ice
2. Neutral Buffered Formalin (10%), Fisher 22-050
3. PBS
4. DMEM + antibiotics
5. OCT compound
6. DMSO sterile
7. Liquid nitrogen vapor
8. 70% Ethanol

Equipment:

1. Nitrile Gloves
2. Disposable gown or Whitecoat
3. Styrofoam box with lid or solvent compatible ice bucket
4. Forceps
5. Biohazard bags
6. Disposable #10 blade and scalpel
7. Media tubes (x4, will need 5 if collecting bone marrow core)
8. Tissue Cassettes (x8, will need 9 if collecting bone marrow core)
9. Tissue Culture Plate
10. Whirly specimen bag for OCT storage
11. OCT mold (x4)
12. Cryovials (x4, will need 5 if collecting bone marrow core)
13. Blood collection tubes (3 EDTA purple top and 1 Serum red top)
14. Bone Marrow Aspirate tubes (3-4 EDTA purple top)

SAFETY WARNINGS

Health and Safety:

1. Safety glasses or goggles, proper gloves, and a lab coat required. The area should be adequately vented and a lab mat placed underneath all solutions and tissue.

1 Specimens to be collected:

1.) Skin (peri-incisional) 2x1cm
2.)Liver Tissue 1cm3
3.) Liver Tissue 1cm3a abdominal (omentum) and/or subcutaneous, 2 x 2cm3
4.) Bone Marrow
5.) Blood

Skin/Liver/Fats collection

2 Skin/Liver/Fats

Ideally, a 2 x 2 cm3 piece of each tissue will be collected by the operating surgeon and passed off the field in specimen containers appropriately labeled. Bring small cooler with ice to the OR. In cooler have four separate 30 ml conical tubes filled with 10 ml of DMEM Media and labeled with tissue type (skin, subcutaneous fat, intra-abdominal fat, liver). Retrieve each tissue type from surgeon or nurse and transfer to DMEM tube immediately.

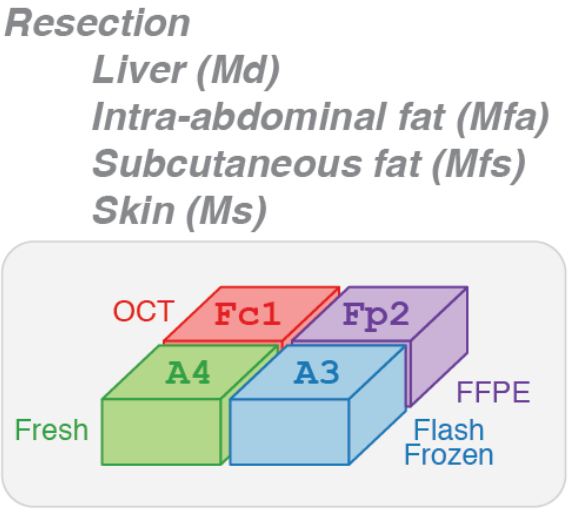
3 After tissues are retrieved from the OR and immediately brought to the Biospecimen Core laboratory where it is subdivided and stored according to the method of preservation

4 The Pieces are named as follows: tissue collected under the SenNet protocol, the collection name will be SN123H1, with 123 an increasing number, H for healthy, and 1 indicating first collection. The following specimen names are used:

- Ms1: Skin
- Md1: Liver
- Mfa1: Intra-abdominal fat
- Mfs1: Subcutaneous fat

Following this, the specimens are divided as shown in Figure 1 and named as (using skin as an example):

- SN123H1-Ms1Fc1: OCT
- SN123H1-Ms1Fp2: FFPE
- SN123H1-Ms1A3: Flash Frozen
- SN123H1-Ms1A4: Fresh. Alternatives:
- SN123H1-Ms1Fs4 - Formalin-fixed, stained, and processed for light sheet
- SN123H1-Ms1Fc4 - OCT



Example: SN123H1-Ms1Fp2

Figure 1

5 Each tissue type will be subdivided into four equal pieces for downstream analysis. First piece of each tissue will be placed into OCT, see step 6.

6 OCT: tissue is placed into cryomold (Cryomold, Standard, ID-0.95 in., depth-0.26 in., catalog # 4728, Tissue-tek) and OCT solution (Tissue Plus O.C.T. compound, catalog # 4584, ThermoFisher Scientific) is applied to completely cover the tissue and fill the mold, it is then immediately placed on to a metal block that is surrounded by dry-ice. The cryomold containing the tissue will slowly freeze solid and preserve the integrity of the tissue within. Once each cryomold has frozen completely then place it into the minus 80 to be stored. Samples are recorded and tracked with OpenSpecimen.

7 Second piece of each tissue type will be placed into FFPE to be made into paraffin blocks and H&E staining. See step 8.

8 FFPE: tissue is placed into the well of a 24 well tissue culture plate, oriented, and flattened, 10% Neutral buffered formalin (NBF) is then added to completely cover the tissue. When all FFPE samples are taken and prepared, the 24 well plate is then placed into a 4o C refrigerator. After 24 hours the NBF must be replaced with 70% Ethanol and tissue is then placed into specialized cassettes (Histosette II Tissue cassettes, catalog # 15182701A, ThermoFisher Scientific) between two blue biopsy pads (Biopsy Foam Pads, 30.2x25.4x2mm, catalog # 22038221, ThermoFisher Scientific) and returned to the refrigerator for eventual processing into paraffin blocks. Specimens in cassettes can be left in 70% ethanol for up to 3 months. Tissues that remain in 10% NBF longer than 24 hours can be subject to antigen degradation that can seriously affect the integrity of downstream assays, e.g. antigen based immunohistochemistry stains, and high dimensional spatial assays (CODEX, Spatial transcriptomics). Paraffin blocks should be stored in the minus 80, catalogued, and tracked with OpenSpecimen.

9 Third piece of each tissue type will be Flash frozen in Liquid Nitrogen. See step 10.

10 Flash Frozen: Tissues are placed into a labeled cryovial and immediately plunged into a dewar of liquid nitrogen. Once tissue is frozen, samples can then be placed into a minus 80 freezer in the appropriate box for storage, cataloguing and tracking with OpenSpecimen.

11 Fourth piece can be used for either fresh sequencing or duplicate of other method of preservation. Currently we do a second FFPE sample as the fourth alternative (labeled as SN123H1-MS1FP4).

12 What to bring to the OR.

Bone Marrow OR Collection Procedure.docx

13 Approxiamtely 20 ml of bone marrow will be obtained from the iliac crest in the OR.
Biopospecimen team will obtain specimen from surgical team that consists of liquid aspirate and a core of biopsy material. The bone marrow core material will be transported in PBS and conveyed to the laboratory as quickly as possible. The liquid aspirate will be placed into blood collection tubes with sodium heparin anticoagulant (green top).

14 The bone marrow core will be dissected into two equal pieces, 1.) OCT-Placed into cryomold, covered in OCT and frozen on a metal block surrounded by dry ice, and 2.) FFPE- dropped into formalin and processed exactly the same as above FFPE tissue specimens (bone marrow core mostly will be diameter of 3.048mm and length of ~2cm)

15 The naming scheme will follow as with the above tissue specimens:

- SN123H1-Ma1Fc1: OCT
- SN123H1-Ma1Fp2: FFPE
- SN123H1-Ja1Jp1_1: Bone marrow EDTA Plasma aspirate, first aliquot
- SN123H1-Ja1Jn2_1: Bone marrow BMMC aspirate, first aliquot

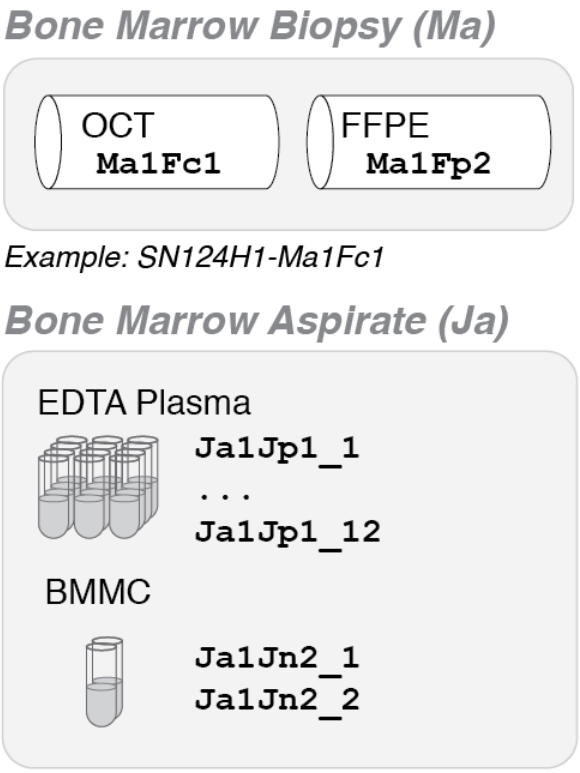


Figure 2

16 Bone Marrow Aspirate is processed the same as peripheral blood. See below for processing instructions.

Blood Processing

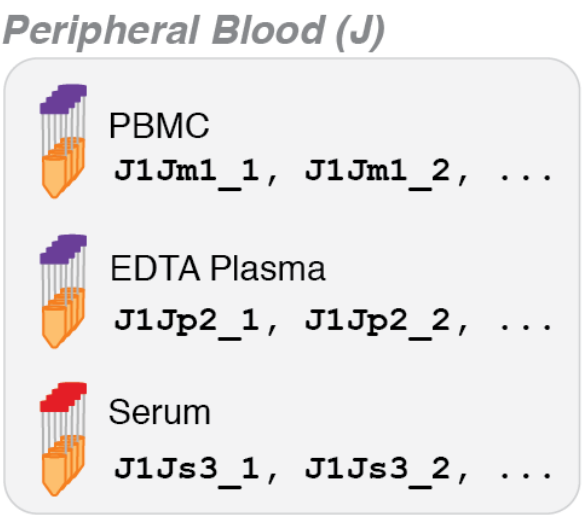
17 Between 20-30 ml of blood to be collected along with tissues. Bring three purple top blood collection tubes (EDTA tubes) and one Red top tube (serum collection tube). All tubes in a bio-specimen bag and carried inside the same cooler as the tissues. Will need to talk with the Anesthesia team inside OR for blood collection as they will take blood when the IV is started in the OR after patient is asleep. Surgeon will not be involved with the process more than likely.

18 Once the blood is collected in the tubes, it is transported back the the lab to be processed. See steps below.

19
1. Counterbalance serum and in centrifuge and spin at 3000g for 5 minutes
2. Prepare ficoll for blood processing: pour ficoll into 50 mL conical tube equal to amount of blood collected (3 tubes ~ 25 mL)
3. Use serological pipette and slowly pipette the blood on top of the ficoll, allowing it to run down the side of the tube and set on top of the ficoll

4. Remove serum from centrifuge, counterbalance ficoll/blood conical tube and spin at 400g for 30 minutes
5. Pipette 0.5 mL of serum into labeled screw-top tubes and 1.5 mL urine into labeled screw-top tubes and set on ice
6. When ficoll/blood is finished spinning, remove plasma layer using serological pipette and put in 15 mL conical tube, careful not to pick up the buffy coat
7. Pipette 1.6 mL plasma into Eppendorf tubes and spin in Eppendorf centrifuge at 16000g for 10 minutes
8. After spinning, pipette 1.5 mL plasma into labeled screw-top tubes, careful not to pick up the pellet, and set on ice
9. Remove buffy coat with serological pipette from ficoll/blood mixture and add to 50 mL conical tube
10. Fill 50 mL conical tube to the 50 mL mark with DPBS and spin at 400g for 10 minutes
11. After spinning, discard DPBS in waste, gently break up pellet with 1 mL DPBS
12. Again fill 50 mL conical tube to the 50 mL mark with DPBS and spin at 400g for 10 minutes
13. After spinning, discard DPBS in waste, gently break up pellet with 1 mL DPBS
14. Using serological pipette, fill 50 mL conical tube to the 10 mL mark and gently pipette up and down to mix
15. Add 15 µL of DPBS/buffy coat mixture to a 15 mL conical tube
16. Add 15 µL of trypan blue to the 15 mL conical tube
17. Again fill 50 mL conical tube to the 50 mL mark with DPBS to the remaining DPBS/buffy coat solution and spin at 400g for 10 minutes
18. While DPBS/buffy coat solution is spinning, add 15 µL buffy coat/trypan blue solution to hemocytometer and obtain cell count
19. Count 4 small squares
20. Multiply this number by 4
21. Double this number
22. Move decimal over one spot to the left
23. Divide number by 5
24. The resulting number is how many milliliters of freezing media are needed to add to the buffy coat conical tube after the last spin
25. After the third spin, discard DPBS from DPBS/buffy coat conical tube
26. Use 1 mL freezing media to gently break up pellet
27. Using serological pipette, add amount of freezing media needed from the cell count, but subtract one from the pellet-breaking process
28. Gently pipette PBMC/freezing media solution up and down to mix
29. Pipette 1.0 mL of PBMC/freezing media solution into labeled Nalgene tubes
30. Place serum, and plasma tubes in -80, along with PBMCs in the Mr. Frosty

20 Label as follows:



Example: SN124H1-J1Js3_3

Figure 3