Wake Forest University Health Sciences
Manual of Procedures (MOP)
Biospecimen Collection and Processing
Version 2.0

9/13/2023

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For Buck Institute SenNet TMC
"Senescent cell mapping, identification and validation for human somatic tissues"

1. BACKGROUND & OBJECTIVES

This protocol is part of a larger multi-center study operating under separate protocols to support the acquisition of blood, urine and muscle tissue only at Wake Forest that will go to the related Cores at the Buck Institute as part of their Cellular Senescence Network: Tissue Mapping Center U54 grant. The long-term goal of the Biospecimen Core is to facilitate a blueprint of cellular senescence in healthy human ovaries, breast, and muscle across an aging continuum. The overall objectives are to procure, curate, and validate human ovarian, breast, and muscle tissues and associated bio fluids (follicular fluid, plasma, and urine) and demographic and clinical health data. Tissues are analyzed through the Biological Analysis Core using a suite of sophisticated transcriptomic, proteomic, and morphometric tools to resolve cellular senescence at the single cell level. These tissues and data are shared with other SenNet Tissue Mapping Centers (Schematic). Mapping senescent cells in ovary, breast, and muscle will provide the first insights into cellular senescence differences between reproductive and somatic tissues, elucidate ubiquitous and tissue-specific signatures of cellular senescence, and reveal the burden of cellular senescence during aging.

This MOP only involves the methods associated with obtaining muscle, blood, and urine along with demographic, clinical, health, and functional data. The aims are modified to only include those related to operations specific to Wake Forest specimen and data collections, not the entire multi-center study.

Objectives

Aim 1: Identify sources of healthy human muscle tissues and associated bio fluids for senescent cell mapping. Muscle biopsies will be collected longitudinally 3 years apart from healthy males and females (N=88), balanced for young (20-30y) and old (>70y) ages; matched urine and plasma samples will be collected along with demographic and clinical health data.

Aim 2: Develop a pipeline to procure, curate, and validate human muscle tissues and associated bio fluids for senescent cell mapping. We will collect and send fresh and frozen specimens which are bar-coded, processed, and stored in locked and alarmed freezers. All accompanying data is entered and stored in a dedicated and secure Biospecimen database. Certified pathologists map cellular senescence in healthy human tissues as validated by gross and microscopic evaluation of all tissues.

Aim 3: Enable distribution of human muscle tissues and associated bio fluids for senescent cell mapping through the Biological Analysis Core and SenNet Tissue Mapping Centers. All tissues are obtained under an IRB-approved protocol following informed consent to enable sharing across SenNet Tissue Mapping Centers. The Biological Analysis Core will use advanced technologies (single nuclei Seq, spatial transcriptomics, and mass spectrometry) to evaluate cellular senescence in these samples.

2. STUDY OVERVIEW

A brief overview of the steps involved in completing the Biospecimen collection is outlined here. An assessment schedule schematic follows on the following page. More detail regarding each step is provided in the sections below.
In addition to participant and collections overview, this specific Biospecimen MOPs are provided for:

2.1 Study Description
Participants are screened initially over the telephone to determine eligibility for Biospecimen collection. Major exclusions include the use of medications that affect bleeding, bruising, and/or platelet function, history of a bleeding disorder or a serious allergy to lidocaine. Medications are reviewed with participants during their screening to ensure no exclusion medications were missed during the telephone screening process. Also, during the screening assessment, participants will have physical performance and cognitive testing performed and then again at follow up.

Biospecimens will be collected in the morning after an overnight fast. Biological material obtained from study participants include blood samples obtained for whole blood, plasma, and serum; muscle tissue associated adipose and connective tissue obtained from the vastus lateralis, and urine collected 4 days post D3 creatine dose. Some of the blood will be used to measure clinical labs such as metabolic panel and HbA1c to better characterize participant health. Participants will self-report personal information on paper survey forms including personal demographic data, race, health information, and psychological and social data. Results will be recorded on a paper form or in a computer database.

Participants are instructed to avoid eating or drinking anything except water for at least 10 hours before the tissue collection appointment. Participants are given a snack after their appointment. Participants are given instructions for the muscle biopsy prior to their scheduled Biospecimen collection appointment and will be given instructions for care after the procedure. Two to three days after Biospecimen collection, participants will be contacted via telephone to check on the participant and in the rare situation that an adverse event occurs, an adverse event form will be completed.

The creatine pills and instructions will be provided to participants prior to the six urine collection time points (every 7 months) to ensure they have time to take the pill (~4 days) prior to the urine collection.

Setting
All participants will be instructed to come to the J. Paul Sticht Center on Aging and all tests and procedures will be conducted there and within the Geriatric Research Center.

Subjects selection criteria
We will recruit and enroll 88 healthy, young (20-30 years) and older (70+ years), community-dwelling men and women (50% each) who meet the additional inclusion/exclusion criteria listed below. These criteria are in place in order to maximize safety of study participants and maximize generalizability.

Inclusion Criteria
1) Healthy subjects will be 20-30 years of age or over age 70.
2) They will be able to sign informed consent.
3) They will be on no prescription medications that could significantly affect their heart or cardiovascular system or make it unsafe for muscle sampling.

Exclusion Criteria
Subjects will be excluded if they are hypertensive. They will be excluded if screening reveals any underlying medical condition that would exclude them as “healthy” or their ability to have a muscle biopsy (i.e. on anti-coagulants or allergic to lidocaine). Subjects will also be excluded if they regularly take any medication that could affect the cardiovascular system or medications that indicates they may be treated for an excluded chronic disease (except for stable blood pressure and cholesterol management). Subjects will be excluded if they anticipate being unable to complete all scheduled assessments including 7-mo urine collections and return clinical visits with biospecimen collections at

3-years. See the table below for full inclusion and exclusion criteria as well as the source of that information.

Criteria	Inclusion	Exclusion	Assessment
Age	20-30 years OR 70+ years (target 50% women each category)	<20 years OR 31-69 years	Self-report (phone)
Housing status	Community-dwelling	Resides in assisted living or skilled nursing home	Self-report (phone)
Obesity status	Must be 18-40 kg/m2	BMI <18 or >40 kg/m^2	Self-report (phone) +Confirmed at screening visit
Physical function status	Unimpaired	• Dependent on cane or walker • Inability to walk independently • Needing assistance with any activity of daily living	Self-report (phone)
Cognitive function status	Unimpaired	• History of mild cognitive impairment or dementia • Cognitive impairment (score <22) on Montreal Cognitive Assessment (MoCA)	Self-report (phone) Conducted at screening visit
Psychiatric status	Stable	• History of untreated and/or unstable clinical psychiatric disorder other than depression or anxiety • Psychiatric hospitalization within the past year	Self-report (medical history at screening visit)
Smoking/ alcohol use	Non- or moderate- use	• Excessive alcohol use (>7 for women or >14 for men drinks/week) in the past month • Use of >1 tobacco product per day or 4 per week, or vaped more than once a week within past year	Self-report (phone)
Nutritional status	Weight stable	• Weight loss or gain ≥10% in past 6 months	Self-report (phone)
Orthopedic status	Unimpaired	• Severe arthritis, fracture, chronic injury, or other musculoskeletal disorder that prevents walking independently	Self-report (phone) or at screening (medical history)
Severe arthritis and inflammatory conditions	Unimpaired	• Severe arthritis that limits normal movements and activities • Rheumatism or ankylosing spondylitis	Self-report (phone) or at screening (medical history)
Communicable diseases	Free of chronic communicable disease	• HIV-1/HIV-2 • HBV • HCV • Medications to treat or control chronic viral infection (e.g. interferon, antivirals, antiretroviral therapies – NRTI's, NNRTI's, PIs, INSTIs, and enhancers)	Self-report
Co-morbidity/health history	Free of major chronic disease and life threatening conditions	• Osteoporosis • Uncontrolled hypertension (systolic >140 OR diastolic >90 mmHg) upon repeated assessments (up to 3 times)). (controlled by stable blood pressure medication OK) • Type 1 diabetes (or insulin use) • Type 2 diabetes (or HbA1c >6.5% in EMR if available) • Dialysis or kidney disease • Liver disease or cirrhosis of the liver • Stroke, heart attack, heart failure	Self-report Self-report or screening BP Self-report

		hospitalization, or revascularization procedure within the past year; New York heart failure Class >2; uncontrolled angina • Pacemaker • COPD requiring oxygen use • Progressive neurologic disease (e.g., Parkinson's, ALS, MS) • Other diseases suggesting a life-expectancy <3 years • Cancer requiring treatment (except non-melanoma skin cancers) within 5 years • Major, uncorrectable vision or hearing loss that may limit participation	Self-report
Bleeding, clotting, and wound healing		• Bleeding disorder low platelet count, hemophilia, or a clotting problem • Impaired wound healing • Foot or other skin ulcer	Self-report
Medication use		• blood thinners, such as Aspirin, Coumadin, Eliquis, Plavix, Pradaxa, Warfarin, Urokinase, or Xarelto • If taking aspirin - unable / unwilling to stop taking aspirin for about a week • Medications for diabetes or prediabetes • Medications to treat or suppress communicable diseases like HIV, HBV, HCV	Self-report
Drug allergy to numbing medication		• Allergic to Betadine, Novocaine, or Lidocaine, or any other numbing medication	Self-report
Non-compliance		Unable/unwilling to: • Provide own transportation to study visits • Commit to study protocol, including 6-mo at home urine collection • Adhere to data collection visits	Self-report
Research participation		• Current participation in another intervention research study • Planned out-of-town trip greater than 6-months • Planned move from area within 3-years and unable to return to clinic for follow-up visits • Unwilling to provide informed consent • Judged unsuitable for the study for any reason by research team	Self-report Principal Investigator / Medical Safety Officer

Sample Size

Recruitment will be coordinated and facilitated by the Clinical Research Core recruitment staff of the WFU Pepper Center which uses trained recruiters and a web-based data-tracking system to monitor recruitment and recruitment strategies. We will recruit 44 men and women, ages 20-30 years and 44 men and women, ages 70 and up, with equal (50%) enrollment of each sex. There will be no upper age limit as an exclusion as long as individuals are healthy and meet other eligibility criteria. We will target recruitment a goal of 20% of participants who identify as Black and <5% as Hispanic/Latino.

2.2 WF Clinical Site - Study Assessment Schedule

Study Assessments	Telephone Screen	Screen Visit	Baseline Visit	7-mo Urine	3Y Follow-up Visit 1	3Y Follow-up Visit 2
Visit Code	**TS**	**SV1**	**BV**	**UV1-UV4**	**FV1**	**FV2**
Phone screen/phone consent	X					
Informed consent/HIPAA form, demographics.		X				
Montreal Cognitive Assessment (MoCA)		X			X	
Vital signs, BP/ pulse, height /weight /waist circumference		X			X	
Medical history and medication checklist		X			X	
Physical performance: Expanded Short Physical Performance Battery, 400 meter walk (fast), Grip strength		X			X	
Blood draw and clinical labs^			X			X
Skeletal muscle biopsy			X			X
D3-Cr dose*		X		X	X	
D3 Cr urine collection**			X	X		X
Weight				X		
Health and medication change checklist				X	X	

^clinical labs include complete metabolic panel and HbA1c

**Participants will take D3-creatine dose at home; at 7-mo time points study personnel will call the participant to remind them to take the dose and review procedures for at-home urine collection, if needed.*

***At 7-mo visits urine will be collected by participants at home or in the clinic. Following home-collection, the urine will be delivered and processed at clinic prior to shipment to Dr. Evans' laboratory at UCB.*

3. PARTICIPANT PRESCREEN AND PROCEDURE INSTRUCTIONS

3.1. Screening

We will use screening procedures to determine if potential participants are interested in taking part in the study. This is done through an initial Telephone Screening call where several eligibility requirements are addressed, including those that are specific for the tissue collection.

In general, participants are asked if they are regularly taking medications that strongly affect bleeding, bruising, or platelets. These medications include:

1. Clopidogrel, Ticgrelor, or Pragruel (also known as Plavix, Brilinta, Effient)
2. Warfarin (also known as Coumadin)
3. Apixaban, Rivaroxaban, Dabigatran, Edoxaban (also known as Eliquis, Xarelto, Pradaxa, Savaysa)
4. Aggrenox (also known as Dipyridamole and Persantine)
5. Ticlid (also known as Ticlopidine)
6. Agrylin or Xagrid (also known as Anagrelide)

If a participant is regularly taking any of the above medications above, the participant is not eligible.

If the participant reports taking prescribed aspirin, procedures are in place to obtain information about why aspirin is being taking and when aspirin was first prescribed to the participant. Participants are asked if they previously withheld taking aspirin prior to a procedure, such as a dental procedure. A Medical Safety Officer or Study Physician will review aspirin use and follow-up with the participant and the prescriber of the medications (primary care physician, cardiologist, etc.) as appropriate and if possible, establish an appropriate plan for the participant to withhold aspirin prior to the tissue sampling procedure. Those participants who are taking aspirin without a prescription are asked if they are willing to withhold aspirin prior to the sampling. Participants are reminded of this plan to withhold aspirin during study visits and with reminder phone calls prior to their scheduled appointment.

Other medications that are asked on the Telephone Screening include the following:

1. Daily non-steroidal anti-inflammatory drugs (NSAIDs) (also known as ibuprofen
2. [Advil, Motrin]; naproxen [Aleve]) (Only excluded from the study if they are not
3. willing to withhold these meds for 3 days prior to tissue collection)
4. Daily steroids (by mouth) (including Prednisone, Prednisolone, Betamethasone,
5. Dexamethasone, Hydrocortisone, Methylprednisolone, Deflazacort or Budesonide.)

If the participant reports taking any of these medications, they will be excluded from the tissue procedure and the study at the time of screening. These are reviewed again before the procedure.

If a participant indicates that they do not know what medications they take, this may be a sign of cognitive problems. Participants who say don't know to the medication screening questions or refuse to provide answers will be excluded from the procedure. Those with any allergies to lidocaine is excluded from the procedure. The study physician will review those cases where the participant are told that they have a bleeding disorder or clotting problem to determine if they should not be eligible for the study.

3.2. Participant instructions for procedure

Participants should receive the Muscle Tissue Collection Instructions sheet before the tissue collection procedures. Participants will also receive a sheet with frequently asked questions about muscle collection, and they can share this with family members who may be concerned about the procedure.

The instructions provide a brief description of the tissue collection procedures. Participants are instructed to call the clinic with any questions. The participant instructions for preparation for muscle tissue collection are described below.

1. Fasting: Do not eat or drink anything except water for 10 hours before your appointment time until after the tissue collection has taken place. At that time, you are provided with a snack.
2. Take your prescription medications as usual with water EXCEPT:
    a. If you take an oral medication or insulin for diabetes, bring it with you to take right after the sampling procedure.
    b. If you were asked to withhold aspirin, stop taking it three days before the procedure.
3. If you have pain or cold symptoms, it is ok to take Tylenol™ or Extra-strength Tylenol™ (acetaminophen) in the 1 week before the procedure.
4. Do not engage in strenuous exercise for 48 hours before the procedure.
5. Prolonged fasting prior to your visit is not advised. Please have an evening snack if there are 12 or more hours between your last meal and your appointment time.
6. Please drink plenty of water while fasting!
7. It is recommended that you bring loose fitting shorts or a skirt to wear during the procedure.

Participants will also be given instructions for after the tissue collection procedures. They will be instructed to limit their activity for the rest of the day and to not take a shower for 24 hours or bathe in a tub for 72 hours after the procedure.

3.3. Handling participants who are extremely apprehensive about having tissue collected

Remember that participants can opt out of the muscle collection at any time. If a participant has concerns, it may help to explain to the participant that every effort will be made to make sure they are as comfortable as possible. If a participant expresses concerns that cannot be easily addressed at the time of the tissue collection, please call the person who will be obtaining the tissue collection to speak with them. Do not under any circumstances force the participant to have a muscle biopsy.

3.4. Pre-tissue collection review of eligibility criteria

Although participants are pre-screened for tissue collection eligibility at the time of the Telephone Screening, it is important to review all eligibility criteria for the muscle biopsy on the day of the procedure. Before reviewing eligibility, make sure the participant is feeling well. If the participant is not feeling well, reschedule the muscle tissue collection procedure.

If a participant is regularly taking a new medication (or one that was not noted during the eligibility telephone screener) that makes them not eligible for the tissue collection, such as Coumadin, Plavix, Aggrenox, Ticlid, Agrylin, Xagrid, Aricept, Namenda, Exelon, or Razadyne, the muscle tissue sampling should be canceled. These participants are not rescheduled and they will not provide a tissue sample.

If a participant is regularly taking a new steroid by mouth (or one that wasn't noted during the eligibility telephone screener), such as Prednisone, Prednisolone, Betamethasone, Dexamethasone, Hydrocortisone, Methylprednisolone, or Deflaxacort, the participant is no longer eligible for muscle tissue sampling.

Ask the participant if they have taken aspirin or aspirin-containing products or Aleve, Ibuprofen, Motrin, or any other anti-inflammatory medications in the past 3days. If so, alert the medical safety officer or study physician who will be obtaining the muscle tissue. Depending on how much aspirin or other NSAID the participant may have taken, the physician may decide to reschedule the muscle tissue collection. Indicate if study physician recommends that participant continue with the procedure on the data collection form.

Ask the participant if they have avoided strenuous activity in the last 48 hours. If they have performed any strenuous activity, their biopsy should be rescheduled. Take participant's seated, resting blood pressure. Elevated Blood Pressures will be classified as Alert Level 1 or Alert Level 2.

1. Alert Level 1: Systolic >180 or <90mmHg; Diastolic >110mmHg Level 1 BP alerts will lead to cancelation of the tissue sampling. Participants will be considered on blood pressure hold and may be reconsidered for tissue sampling after they are evaluated (and cleared) by their primary providers. When a Level 1 alert is observed document the alert in SenNet data system.
2. Alert Level 2: Systolic 160 - <180 or 90-100mmHg; Diastolic 100 - <110 mmHg. For level 2 BP alerts, the CPET session may continue at the discretion of the study physician (or the physician delegate). When a Level 2 alert is observed, document the alert in SenNet data system..

Ask participant the date and time they last ate or drank anything other than water. Record the time on the data collection form. If the participate ate or drank anything other than water within 10 hours of the sample collection, the examiner should record what the participant ate and/or drank and if the food or drink included caffeine. The study physician should review the case. Taking into consideration the degree of infraction and the likelihood of the participant rescheduling, the study physician may approve collection of tissue even though the participant did not fast for 10 hours. This approval should be documented on the data collection form.

In general, we should use the right side unless there are contradictions. Be sure to note which side you need to access before asking the participant to lie down on the table and position the participant accordingly.

Have the participant change into a gown and lie comfortably in a reclined position with both legs outstretched.

Ask participant if they are allergic to latex, and if so, use alternative material gloves.

4. CLINICAL STAFF PERSONAL PROTECTIVE EQUIPMENT AND SANITATION

Staff members will refer to Wake Forest institutional policies regarding work with human participants and the handling of biological samples to ensure the safety of both clinical staff and study participants. These policies should include details about proper use of personal protective equipment and hand washing for clinical staff, disinfection of work areas and equipment, as well as proper disposal of contaminated materials (such as needles and medical consumables).

5. BIOSPECIMEN ID LABELS

Cryotubes labels are made by the staff at the Wake Forest Baptist clinical site Biogerontology Laboratory using Freezerworks labels. Then the samples are shipped to the Buck Institute SenNet Biospecimen Core for Bio-banking.

6. URINE COLLECTION PROTOCOL

6.1 Preparation of Participants for Urine Collection

Providing the Dose

Provide the dose at the screening visit or other contact prior to a scheduled clinic visit for urine collection. Participants should be given their dose in the provided snap cap pharmacy vial (6 dram). Sites may write the participant's name and date and time to take the dose on the vial label prior to giving the dose to the participant.

If mailing the dose to the participant, send the participant the dose, instruction sheet, collection container and biohazard bag (if collecting the sample in clinic a collection container and biohazard bag is not required) so that the participant will receive it at least 3-6 days before their scheduled clinic visit. The dose should
be packaged in a snap cap pharmacy vial (6 dram). Sites may write the participant's name and date and time to take the dose on the vial label prior to mailing the dose to the participant.

A reminder phone call the day of or the day before the participant should take the dose should be made to remind the participant about instructions for taking the dose and timing.

Instructions for taking the dose

Provide instructions regarding taking the dose.
The instructions inform the participant:

- The dose may be taken with or without water or other liquid.
- The dose may be taken with a meal, or between meals.
- The dose must be taken 3-6 days before the clinic visit. Sites will call the participant the day before the dose should be ingested as a reminder. A reminder call earlier than the day before is also acceptable.
- The participant should write down the time and date the dose was taken on the dose administration worksheet. This information should be recorded on the form when the participant comes into the clinic.

Day Of

1. Urine is collected before venipuncture; preferably as early in the visit as possible. The labeled creatine dose will not interfere with any other assays that may be competed in the collected urine.
2. Encourage participants to stay hydrated (water only) even while fasting for the visit. However, do not collect samples after acute fluid load (>24 oz.) or after participant exertion.
3. Participants having difficulty producing a urine specimen are offered a glass of water.
4. A sample provided before 3 days (72 hours) or after 6 days (144 hours) will be considered invalid. The participant should be instructed not to eat or drink anything EXCEPT WATER for at least eight hours on the day before the urine collection. Tea, coffee and other liquids are not allowed.
5. The urine sample collected should not be the first void. Generally, many participants will be able to provide a 2nd or 3rd void. However, some participants will urinate multiple times overnight; thus, voids after the 3rd void are acceptable if the sample is fasting and collected in the morning before 12:00 Noon.
6. We are collecting a fasting sample for both the creatine dilution urine sample and the samples that will be archived.
7. If the participant attends the clinic visit and says they have not used the bathroom in the morning yet and would be providing a first void, ask the participant to provide a sample that is not the first void.
8. Participants will use the Covidien Sterile Midstream Urine Collection Systems (Fisher Scientific Cat# 14-375-143) for urine collection. Instructions are included in the package for the participant.

6.2 Urine (Creatine Dilution / D3)

The goal of this protocol is to understand whether a new method of measuring skeletal muscle mass, called the “creatine dilution method,” is related to strength, physical performance, falls, fractures, disability and mortality in older adults. This method involves several steps. First, the participant ingests a small amount of a special kind of creatine called deuterated creatine. In skeletal muscle, this special kind of creatine is converted into a special kind of creatinine called deuterated creatinine. Then, a few days after ingesting the deuterated creatine, the participant provides a urine sample. The amount of deuterated creatinine in the urine is determined. From this value we can accurately estimate the participant’s total amount of skeletal muscle. We will then determine if the amount of skeletal muscle is related to strength, physical performance, falls, fractures, disability and mortality.

6.3 Urine Collection

1. Instructions for Participants:
    a. The participant’s privacy are assured.
    b. Orient the participant to the supplies and explain the procedure (Covidien
1. Sterile Midstream Urine Collection Systems have clear packaging for ease in
2. describing contents).
    c. Steps to be followed:
    i. Wash hands before and after voiding.
    ii. Open or remove clothing to make voiding and collection easier.
    iii. Remove the cap from the collection container. Void directly into the container until approximately half full.
    iv. Carefully seal the cap of the container so that it is tight and leak proof.
    v. Bring the urine container to staff member.
    vi. Record if urine is collected and approximate volume.

6.3 Urine Processing

1. Keep urine refrigerated or on ice until processing (sample should be cooled within 15 min of collection).
2. Aliquot and freeze within 1 hour of collection.
3. Gently swirl urine immediately prior to aliquoting to ensure sample is thoroughly mixed.
4. For D3 Creatine, on ice, aliquot 0.5mL of urine into one (1) pre-labeled 2.0mL “Urine D3 Creatine” cryovial. Store at -20°C.
    a. This tube is shipped directly to site of analysis not to the biorepository.
5. For Urine archives, on ice, aliquot 1.5mL of urine into eight (8) pre-labeled 2.0mL “Urine” cryovials. Store at -80°C±10.
6. Do NOT overfill the cryovials. There must be space for the urine to expand when frozen.
7. Double-check caps are securely tightened on filled cryovials.
8. Freeze cryovials in an upright position at either -20°C or -80°C±10.
    a. -20°C for D3 Creatinine cryovial.
    b. -80°C±10 for remaining 8 urine cryovials for archiving.
9. Discard any extra urine.

After processing specimens, please contact the Biogerontology Lab to coordinate with the monthly sample pick-up.

6.4 Urine Supplies

- Dose:
    - Labeled creatine dose in provided pharmacy vial
    - USPS priority mail packaging or first class mail to mail dose (if mailing)
    - Instructions sheet to give to participant
- Collection:
    - Urine collection container (same container as main study urine collection)
    - Biohazard bag (if participant is collecting sample at home)
    - Label for collection container (provided by site)
- Processing
    - One 2.0 mL capped cryovials (Same as from main urine collection: Fischer Scientific#: 12-567-501)
        - Fill with .5mL urine
    - -20°C freezer
    - Labels for cryovial (provided by Wake Forest Baptist CRU processing lab)
- Shipping material (to send the specimen to the laboratory)
    - Freezer boxes with 8 x 8 cell (for 2.0 mL tubes)
    - Electonic manifest and hard copy manifest
    - Rubber bands
    - Paper towels
    - Styrofoam shipper and cardboard box
    - Dry ice

7. VENIPUNCTURE BLOOD DRAW PROCEDURE

7.1 Participant instructions for procedure

The participant instructions for preparation for blood collection are described below.

1. Fasting: Do not eat or drink anything except water overnight prior to your appointment time (10 hours) until after the tissue collection has taken place.
2. Take your prescription medications as usual with water.

3. Prolonged fasting prior to your visit is not advised. Please have an evening snack if there are 12 or more hours between your last meal and your appointment time. Please drink plenty of water while fasting!

Participants are given instructions after the blood draw.

7.2 Venipuncture Blood Draw Procedure

1. The participants are asked to dress in a short-sleeve shirt to easily access the blood draw collection sites.
2. The phlebotomy procedure is standardized from a sitting position.
3. The participant will rest for at least 10 minutes prior to the blood draw.
4. After the participant is appropriately positioned, a tourniquet will be placed above the selected vein for a maximum duration of two minutes.
5. The blood draw site is disinfected with an appropriate antiseptic (e.g., 70% isopropyl alcohol wipe).
6. A standard blood collection set is used to draw blood from a vein. The list and number of tubes collected will vary depending on study visit, but order of draw will remain consistent. Below is the Draw order to be followed at every visit:
    a. Red Top tubes
    b. SST tubes
    c. EDTA tubes
7. Under filling the collection tubes will be avoided. For example, EDTA vacutainers must be filled to at least 50% of the fill volume of the tube to avoid a dilutional effect from the additive.
8. After all draw tubes have been filled the needle will be removed, and pressure will be held at the site for at least 30 seconds to minimize the formation of a hematoma. When hemostatic, the site are covered with a pressure dressing.
9. All blood tubes are labeled using established method of labeling.

7.3 Blood Processing Procedures

Blood Biospecimen Collection Overview Table

Tube	Volume (ml)	Category	Storage (# aliquots)	Test	Baseline Visit	Year 3 Visit
Purple K2 EDTA	4	Clinical labs	--	HbA1c	x1	x1
Tiger Top SST	8.5	Clinical labs	--	Panels	x1	x1
Red-top Serum	10	Repository	8×0.5mL	Serum	x2	x2
Purple K2 EDTA-single spin	10	Biomarkers &Repository	8×0.5mL Plasma	Plasma	x2	x 2
Purple K2 EDTA – double spin	10	Biomarkers &Repository	8×0.5mL Plasma	Platelet Poor Plasma	x1	x1

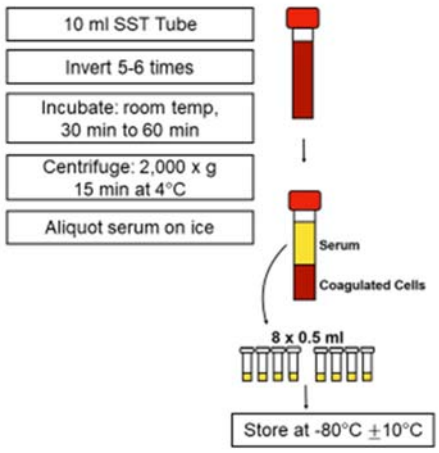
LabCorp spinning instructions:

Collection tube	Test#	Test	Processing	Aliquot/ Transport Vial	Testing Lab	Store at
1 x 4ml K2 EDTA	001453	HbA1c	Send whole blood	Draw tube	Lab Corp	4ºC
1 x 8.5ml SST	322000	CMP	Clot up right for 30min, Spin 3300rpm for 10min @ RT	SST	Lab Corp	Room Temp

7.4 Serum Tubes *(see Figure 2)*

1. 10 ml of blood is drawn into the redtop serum tubes.
2. Directly after blood is added to the tube, the blood should be mixed with clot activator by gently inverting the tube 5-6 times.
3. Place the tube upright to clot at room temperature for at least 30 minutes but no more than 60 minutes (the incubation time will begin at the end of the syringe collection).
4. Centrifuge tube at 2,000 x g for 15 minutes at 4°C.
5. Using a pipette the serum (supernatant) will be carefully removed without disturbing the coagulated cells (when possible, keep the tube on ice.)
   a. Place the pipette tip at an angle against the side of the tube.
   b. If the cellular layer is disturbed, the tube is centrifuged again.
   c. Aliquot 0.5mL of serum, on ice, into 8 labeled 2.0mL cryovials
7. Aliquots will be stored at -80°C±10°C. Storage should occur as soon as possible after aliquot preparation or tubes should be snap frozen.

Figure 2. Processing Red-Top SST Tube for Serum



7.5 EDTA tubes for PLASMA

Overview: Whole blood samples are subjected to two sequential spin conditions. Density gradient centrifugation of the first spin allows to separate the whole blood into three distinct layers: plasma (top layer – aliquot and bank), “buffy coat” (middle layer - used for DNA extraction), and erythrocytes (bottom layer - discard). Both the plasma and buffy coat are store at -80℃ $\pm$ 10°C for future processing, while the red blood cells are discarded.

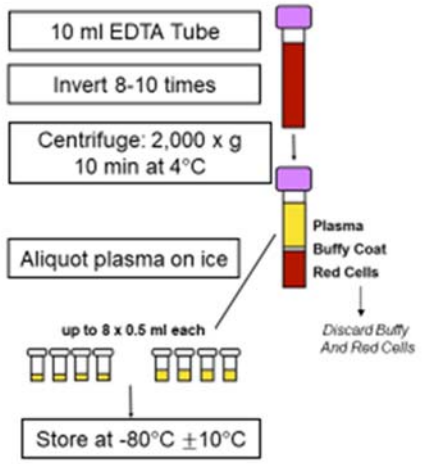
1. Draw 10 ml of blood into the EDTA tube.
2. Directly after blood is added to the tube, the blood will be mixed with the additive by gently inverting the tube 8-10 times.

3. Keep blood at room temperature after collection and process samples within one hour. If the interval between specimen collection and processing is anticipated to be greater than one hour, keep specimen on ice until centrifuging is performed.
4. Place vacutainer tubes containing blood into the pre-cooled centrifuge.
5. Centrifuge tube at 2,000 x g for 10 minutes at 4°C
6. Carefully remove the tubes from the centrifuge without disturbing the sample. The tube will contain three layers:
    a. top – plasma
    b. middle – buffy coat with white blood cells and platelets
    c. bottom – mainly red blood cells (discard)

7.6 Recovery of Plasma – Single Spin *(Figure 3)*

1. Aliquot plasma into cryovials labelled “EDTA Plasma”. Take care not to disturb the leucocyte or buffy coat layer. Ideally, each 10 mL collection tube will yield 8×0.5mL plasma cryovials.
2. Place the cryovials in appropriate freezer storage units.
3. Freeze immediately at -80°C±10°C

Figure 3. Processing 10mL EDTA for Plasma (single spin)

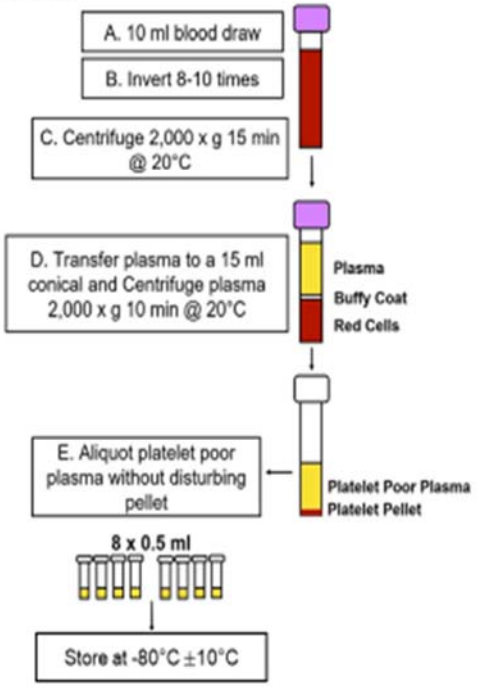


7.7. EDTA Tube for platelet poor plasma (Double Spin Protocol). *(See Figure 4)*

1. Draw 10 ml of blood into a separate EDTA tube.
2. After blood is added directly to the tube, mixed gently by inverting the tube 8-10 times.
3. Process the tube at room temperature for all remaining steps.
4. Centrifuge tube at 2,000 x g for 15 minutes at 20°C. After centrifugation, the tube will contain three layers:
    a. top – plasma
    b. middle – buffy coat with white blood cells and platelets
    c. bottom – mainly red blood cells.
5. After the first spin, the pool plasma is put into a 15ml conical tube and re-spun at 2,000 x g for 10 minutes at 20°C.

6. Aliquot 0.5mL of platelet poor plasma into 8 pre-labeled 2.0mL “Plasma EDTA Platelet Poor” cryovials. Be careful not to disturb the pellet at the bottom of the tube. If the pellet at the bottom is disturbed, the conical tube is centrifuged again.
7. Aliquots will be stored at -80°C±10°C. Storage should occur as soon as possible after aliquot preparation.

Figure 4. Processing 10mL EDTA for Platelet Poor Plasma (double spin)



8. SKELETAL MUSCLE BIOPSY CLINICAL COLLECTIONS

Trained operator using established procedures will perform a skeletal muscle biopsy. A target of 160mg of skeletal muscle is needed for SenNet TMC procedures), with additional tissue for skeletal muscle and adipose tissue archive (~500mg total yield is common).

8.1 Preparation of the biospecimen collection room

The muscle tissue collection should take place in an isolated room. The room should be equipped with all of the necessary supplies and an emergency call button. The processing of the specimen can take place either in the same room as the tissue collection or another room in the clinic that is close by. A separate counter or worktable should be equipped with all of the materials and vials that are used in the tissue handling and processing.

8.2 Muscle Biopsy collection tray

A Mayo stand will contain the material needed for the instrumentation for the tissue collection. The instrumentation will be located on a sterile field. The needles and 60 cc syringe and tubing are sterilized. Listed for guidance only, site established setup may be used.
Tray contents:

- Povidone-Iodine swab sticks
- Steri Strips

- Sterile gauze (4x4)
- Paper tape
- Coban self-adherent wrap
- Scissors
- 2- 5 mm Bergstrom biopsy needle with rubber seal
- 1% buffered lidocaine
- Scalpel handle and blade (#11)
- 2-10 cc syringe
- 1-3cc syringe
- 18g, 22 g and 25g needles
- Extension Line- 33 Inch with lure-slip connection
- 50 cc syringe and tubing for suction
- Sterile gloves of various sizes
- Sterile drape (fenestrated)
- 1-Forceps for blade removal
- Tweezers(110mm long)(#5)
- 1-Spray can of Pain Ease Topical Anesthetic

Other materials should be available in the room (but are not placed on the sterile field):

- Smelling salts
- Dissecting microscope
- Liquid Nitrogen
- Container for Liquid Nitrogen
- Wet ice in a container
- Sharps container
- Pencils/pens
- Non-sterile gloves
- 2- 5 inch straight fine tip tweezers
- 5-Sterile Petri dishes(size 60x15)
- 1- box of Kimwipes
- 12-15 cryovials *(minimum)*- 2 mL Internal Threaded Polypropylene Cryogenic Vial, Self-Standing with Round Bottom
- Cassettes for histology
- Tubes for EM and FIB-SEM, 3.7mL glass shell vials
- Buffers*

*1) FIB-SEM Buffer: 2% Para 2.5% Glut In 0.1M Sodium Cacodylate pH 7.4 from Electron Microscopy Sciences sku #15960-01-1L
2) EM Buffer: Trumps' Fixative: A combination of Sodium Cacodylate, Formalin, and Glutaraldehyde from Electron Microscopy Sciences sku #11750
3) One urine cup with 100ml of 10% buffered formalin to place the histology cassette in the buffer for fixation. Buffer purchased from Fisher Scientific catalog #245-684

8.3 Biospecimen collection rack: labeling and setup
A cryovial rack containing the necessary storage cryovials is set up for each participant with vials according to the priority of the tissue acquisition. Vials will be pre-labeled.

8.4 Preparation for tissue collection
Preparation for tissue collection is performed in the following manner. Early morning, before any participants arrive:

1. Check to make sure that tissue collection trays are properly equipped, including sterilized needles.
2. Check that each tissue cryovial is properly labeled with sample ID labels.
3. Check that the sample processing station is properly equipped with all needed items for processing the tissue.
4. Make sure the tissue collection room is tidy and stocked with extra smelling salts, basin, and disposable wash cloths.
5. Prepare liquid nitrogen for flash (quick) freezing of muscle specimen.
6. Prepare liquid nitrogen and isopentane container for controlled freezing of histology specimen **(Figure 8A below**)
    a. The liquid nitrogen is used to cool the isopentane to near its freezing point. Enough liquid nitrogen is used so that the container of isopentane is sitting atop of the liquid nitrogen (refill with nitrogen as the tissue collection is taking place). By the time the muscle is flash frozen, the isopentane should be near the freezing point as evidenced by some freezing (ice in the bottom). There should, however, still be enough liquid isopentane in the metal cup.
    b. This is recommended to be performed in a hood in the lab not at bedside in a clinic.

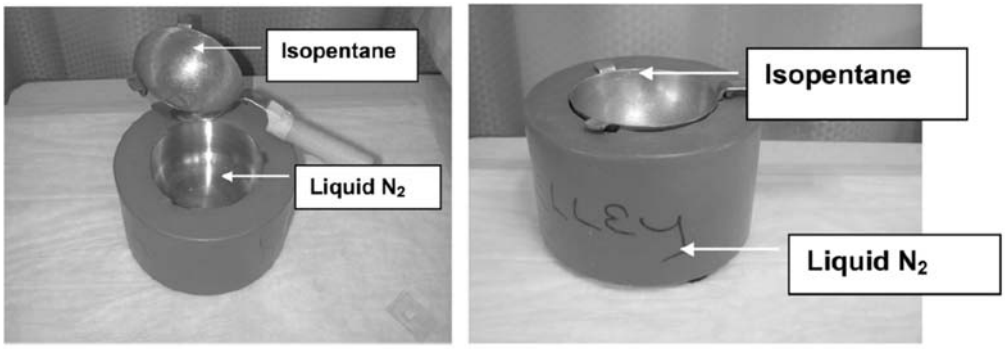


Figure 8A Isopentane / Liquid nitrogen set up.

8.5 Precautions for handling tissue collection specimens

1. In accordance with the OSHA regulations on blood borne pathogens (see OSHA regulations that are kept in the laboratory), the central lab recommends the following laboratory safety protocol for the field center laboratories:
2. Non-permeable lab coats, latex gloves, and face shields are used when handling any blood in any situation where splashes, spray, spatter, or droplets of blood may be generated, and eye, nose, or mouth contamination can be reasonably anticipated.
3. Universal Precautions are followed when handling any tissue products.
4. Contaminated needles and sharps will immediately placed in a puncture-resistant, leak proof sharps container. Never recap or break needles.

8.6 General Information Before Procedure.

1. Personal protective equipment (non-permeable lab coats and double-gloves with at least one latex pair) MUST BE worn for processing.
2. It is possible that not all containers are filled due to problems with the tissue collection or absence of fat or connective tissue in the muscle.
3. During processing, work in the order specified and make as many aliquots as possible while meeting the weight requirement of each cryovial.
4. Record the estimated weight of the tissue samples in each cryovial container.

8. 7 Muscle Biopsy Procedure

8.7.1. Overview

1. The procedures for the administration of lidocaine and the muscle biopsy are performed by a trained operator with a team of support staff signing off on dosage amount administered.
2. The participant will lie supine on the side of the bed, plinth, or chair that best allows clinical staff easy access to the tissue collection sites.
3. The participant will rest supine for at least 15 minutes prior to each biopsy.
4. The muscle biopsy specimen are obtained from the medial vastus lateralis ~15cm above the patella. This is an approximation however, and the practitioner should palpate the vastus lateralis to determine the optimal location for the biopsy (see **figure 8B below**). Right side preferred unless contraindicated.
5. Be sure to document times as the procedure is moving along.

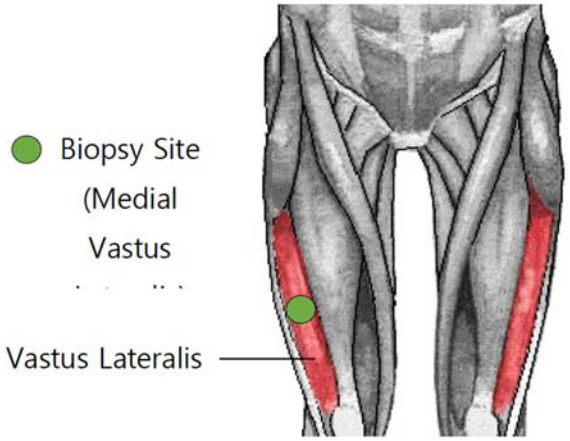


Figure 8B Muscle biopsy location

8.7.2. Anesthesia Administration

1. The skin of the biopsy area is disinfected with an appropriate antiseptic (e.g., povidone iodine or chlorhexidine containing solution) to create a clean surgical field. The study physician may decide to shave the skin if necessary. Place sterile drape over cleaned skin.
2. Document time and amount of administration of lidocaine.
3. The top layer of skin is numbed using a spray of pain ease topical anesthetic.
4. Then to numb the biopsy incision site, an appropriate volume of *2-5cc of 1% or 2% lidocaine* HCL will be delivered by the operator into the skin/subcutaneous tissue to the muscle fascia - but not below the fascia. Wait 3 to 5 minutes and then inject more (~ 10-15cc) lidocaine into the fascia around the muscle in a star pattern. Total amount of Lidocaine administered should not exceed allowable dosage. Lidocaine formula is as follows: maximum allowable dose (mg/kg) x (weight in kg/10) x (1/concentration of local anesthetic) = mL lidocaine.
5. Wait 10 minutes after injection of the lidocaine before proceeding to allow the anesthetic to numb the biopsy site before proceeding to the next step.

8.7.3. Muscle Biopsy Procedure

1. Using a scalpel blade make a small (5 mm) incision in the skin through the subcutaneous fat and through the muscle fascia.
2. A sterile, 5 or 6mm Bergstrom-style biopsy needle will be properly assembled and connected to a 50 cc syringe for suction and the operator from the belly of the muscle acquires a muscle sample.

3. Insert the trocar through the incision, and through the fascia, advancing the trocar into the muscle belly.
4. Once the physician opens the trocar, the assistant will immediately apply suction through a 50cc syringe, drawing tissue into the cutting chamber as the trocar is simultaneously advanced.
5. To maximize tissue yield, rotate the trocar 90 degrees without removing from the leg and repeat the suction procedure two more times for three separate muscle samples.
6. The goal is to obtain 150mg of muscle tissue. If the yield is insufficient after the first insertion of the trocar (<150mg), you may re-insert the trocar up to four additional times (for a maximum of 6 insertions). Each additional insertion is contingent on the operator's best medical safety judgment and ensuring that the participant is comfortable and provides verbal consent.
7. Place tissue on sterile Petri dish.
8. If there is an adequate subcutaneous adipose layer that adipose tissue is collected coincident to biopsy, and participant is comfortable with collection, thigh adipose sample should be obtained and cryopreserved. Note that in some very lean individuals there will be insufficient adipose collection coincident to skeletal muscle biopsy to warrant dissection and storage.
9. A sterile gauze (e.g., 4x4) or similar will be placed over the incision site and firm pressure will be applied with the palm of the hand until hemostatic (typically this is ~5-10 minutes).
10. The incision is closed with each clinical site's preferred technique, such as with sterile liquid bonding agent, steri-strips, sutures, or an adhesive bandage.

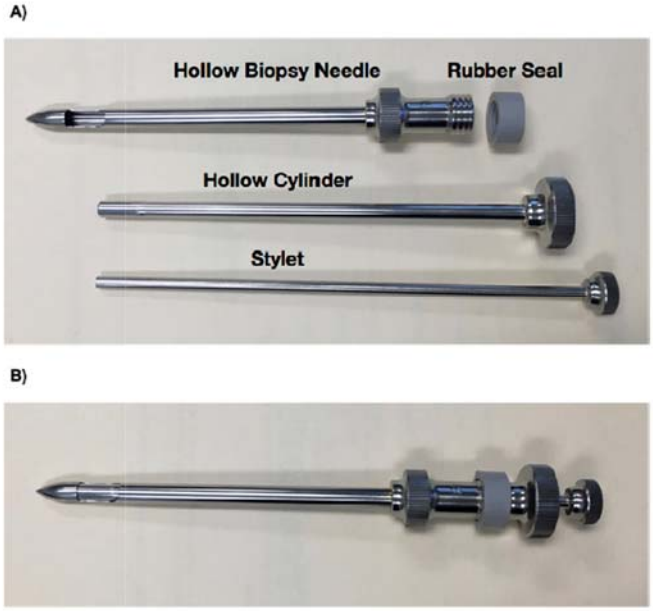


Figure 8C. Bergstrom-style biopsy needle parts (A) and assembly (B).

NOTE: The total amount of time for this procedure is approximately 45 minutes. This includes about 15 minutes of interaction with the study physician for the site preparation and the tissue collection itself; 10 minutes to wait for the lidocaine to take effect, about 15 minutes for icing the area after the tissue collection; and 15 minutes for a snack/recovery. Be sure to offer the snack as soon as possible after the tissue collection.

8.8 Precautions when a participant feels or looks faint following the muscle tissue collection

1. One of the most common adverse events for muscle tissue collection is a vasovagal episode where a participant faints or feels faint.
2. If not already lying down, have the participant lie down with their legs raised onto a pillow or blanket
3. Provide the participant with a basin if they feel nauseated.
4. Have the participant stay lying down until the color returns and they feel better.
5. Place a cold wet cloth on the back of the participant's neck.
6. If the participant faints, use smelling salts to revive by crushing the ampule and waving it under the participant's nose for a few seconds.
    a. If you need to use smelling salts, be sure to complete an Adverse Event Form. Clinic staff should follow-up with the study physician, who will determine the best course of action, including determining if follow-up with a physician is necessary.
7. If the participant continues to feel sick, contact the study physician who will advise you on further action.
8. Always make sure there is a call button or staff member in the room to alert the emergency team if necessary.

8.9 Tissue Collection Discharge Instructions

Before the participant leaves the clinic, the participant who received the biopsy are instructed about care of the biopsy site and made aware of any complications that might occur.

The following instructions are provided to the participant on the Muscle Tissue Collection Discharge Instructions.

- To prevent infection, participants should keep the site clean and dry for at least 24 hours.
- Participants should not shower for 24 hours and should not take a bath for 72 hours.
- Participants should not experience excessive bleeding or bruising. A few drops of blood on the bandage are expected. If excessive bleeding occurs, the participant should call the clinic immediately.
- To prevent bleeding and/or bruising, the bandage is kept on for 72 hours.
- Participant should not do any strenuous activity or exercise for 48 hours after the procedure.
- The participant may take Tylenol for any discomfort as needed.
- If the participant was taking aspirin on a regular basis and asked to stop prior to the sampling procedure, then the participant may begin taking aspirin again on the day after the procedure.
- If there is pain at the tissue collection site, the participant may apply ice every two hours for 20 minutes until the pain subsides.
- Although it is very rare, infection (presenting as redness, pus from the wound, increasing pain) can occur with muscle tissue collection. If the participant develops any of these symptoms, they should call the clinic immediately.
- If the participant has a problem during office hours, they are instructed to call the field center staff. If there is an emergency related to this procedure after normal office hours, the participant should go to the hospital emergency room, and indicate to the hospital staff that a muscle tissue collection was completed.

- Participants are asked to hold anticoagulant medications prior to sampling procedures can resume medications on the day following the sampling procedure.

Participants are given post-procedure instructions for care of the tissue collectionsite, what activities to avoid, and when to report problems. Also, participants should be told that no results will be made available to the participant, but we will contact them to see how they are doing.

8.10 Follow-Up Contact
Although complications after the muscle tissue collection are rare, a follow-up call will be made to assess how the participant is feeling and to assess if there are any complications.

8.11 Adverse Events
We anticipate that adverse events (AE) will be rare. The most common adverse events are likely to be ecchymosis/hematoma, bleeding/oozing at tissue collection site, vasovagal episodes at the time of tissue collection, and pain more than 3 days after the tissue collection.

We do not anticipate infection of the tissue collection site to occur in any participants, although this is a remote possibility. Other possible AEs include reaction to lidocaine or problem related to the bandage or dermabond.

Some potential AEs may occur at the time of the tissue collection and are listed below. The AEs that would not be noted until after the patient leaves the clinic.

- Any allergic reaction to lidocaine is not expected and are considered an adverse event. In the event of a reaction to lidocaine, the physician present will use best clinical judgment to treat the participant based on symptoms and severity.
- Fainting (vasovagal episode) is occasionally expected and are considered an adverse event if smelling salts were needed to revive the participant. Mild cases in which the participant is easily revived without smelling salts do not need to be reported to the IRB; moderate and severe cases, e.g., when reviving the participant is difficult, are not expected and should be reported to the IRB. If a participant faints, a study physician in clinic should examine them.
- Other adverse events are not expected. Any moderate or severe adverse events that do not fit the categories above should be reported. List as many “other events” as needed.
- For each adverse event, the clinic staff should follow procedures outlined in the Safety Protocol including following up the study physician. An Adverse Event Form should be competed. This form will track follow-up with the study physician, the severity of the event, the relationship of the event to the muscle tissue collection procedure, and the action taken The Adverse Event form should be submitted to the SenNet TMC at the Buck Institute.
- When reporting adverse events related to the tissue sampling procedures, please mark the option ‘Biopsy Related’ on the AE data collection form. Then select the appropriate biopsy related reason, using other to capture any biopsy related event that is not a predetermined option. For any skin irritations as a result of bandages, sites should not mark ‘Sink Irritation’ and a Biopsy Related event. As this is specifically related to the tissue sampling, this should be captured only within the biopsy box.

9. SKELETAL MUSCLE PROCESSING

Graphical depiction of workflow and aliquots appended in figures 9A
Default - 12 vials/tubes minimum per biopsy (15 preferred)

Table 9. Skeletal Muscle Tissue Collection & Processing Table for SenNet

	Measure	Amount	Preparation	In-Room Process Order	TMC Priority
Primary Collection & Processing: Target~160mg Flash Freeze & Histo	Cell Culture	50 mg	DMEM media in a 2 ml cryovial @4°C Shipped to BUCK day of on ice packs	1	1
	Histology-DSP-1	40 mg	Frozen fix, Cork, LN2 (aligned)	2	1
	Histology-DSP-2	40 mg	Frozen fix, Cork, LN2 (aligned)	2	1
	Histology-DSP-3	40 mg	Frozen fix, Cork, LN2 (aligned)	2	1
	FFPE Histo Cassette-1	10 mg	10% NBF@24 hrs. to Paraffin embed (aligned)	3	2
	FFPE Histo Cassette-2	10 mg	10% NBF@24 hrs. to Paraffin embed (aligned)	3	2
	FFPE Histo Cassette-3	10 mg	10% NBF@24 hrs. to Paraffin embed (aligned)	3	2
	FFPE Histo Cassette-4	10 mg	10% NBF@24 hrs. to Paraffin embed (aligned)	3	2
	FIB-SEM-1	3-5 mg	Glut./Paraform Fix, 4°C (aligned)	4	3
	FIB-SEM-2	3-5 mg	Glut./Paraform Fix, 4°C (aligned)	4	3
	EM-1	3-5 mg	Trump's Fixative, 4°C (aligned)	5	3
Specimen Archive Larger Yields & SubQ Adipose	Frozen-1	15-20 mg	Flash frozen in LN2	6	4
	Frozen-2	15-20 mg	Flash frozen in LN2	6	4
	Mito Respiration	20mg	Biops buffer on wet ice (aligned)	7	5
	Frozen Archive-1	15-20mg	Flash frozen in LN2	8	archive
	Frozen Archive-2	15-20mg	Flash frozen in LN2	8	archive
	Adipose	10-100mg	Flash frozen in LN2	9	archive

See Figures 9A for graphical workflow and aliquots

9.1 Description of Priority Collections: Flash Frozen.

Upon receiving the biopsy tissue, blot the muscle sample to dryness using a Kimwipes, careful to keep the fibers intact.

1.[Cell Culture] Sterilely select a large piece of muscle ~50mg large and put into in a 2 ml DMEM media cryovial place on ice; ship immediately to BUCK

2.[Histology Cassette x4] Remove a piece of tissue (~10mg) for each histology cassette; Process cassettes in 10% Normal buffered formalin for 24 hours then have tissue paraffin embedded.

3.[Histology-DSP x 3] Select a piece of muscle (~40 mg) showing fibers aligned longitudinally. Transport aligned tissue on gauze on wet ice to the hood in Biogerontology lab to slow freeze in isopentane. Submerge muscle specimen into isopentane **(see Figure 8a)**. Hold submerged and add intact frozen piece in cryovial.

4.[High Resolution Imaging, FIB-SEM x2] Remove two sections (~3-5 mg each) for Focused Ion Beam Scanning Electron Microscopy (FIB-SEM)/Cryo -FIB. Cut longitudinally under the microscope. Place each into their own vial the FIB/SEM buffer previously aliquoted and place back into wet ice. Store tubes (with FIB/SEM buffer) at 4°C until shipment.

5.[High Resolution Imaging, Electron Microscopy x1] Remove a ~3-5 mg piece for electron microscopy (EM). It is best if fibers are intact longitudinally and only 3 to 4 fibers deep. Place the EM section into Trump's Fixative previously aliquoted and place back into wet ice. EM samples are stored indefinitely in a 4°C refrigerator.

9.2 Description of Archival Collections for Larger Yields or Adipose Tissue.

See Figures 9A for graphical workflow and aliquots

If there is still muscle biopsy sample remaining, please prepare as detailed below (See Table 9 above).

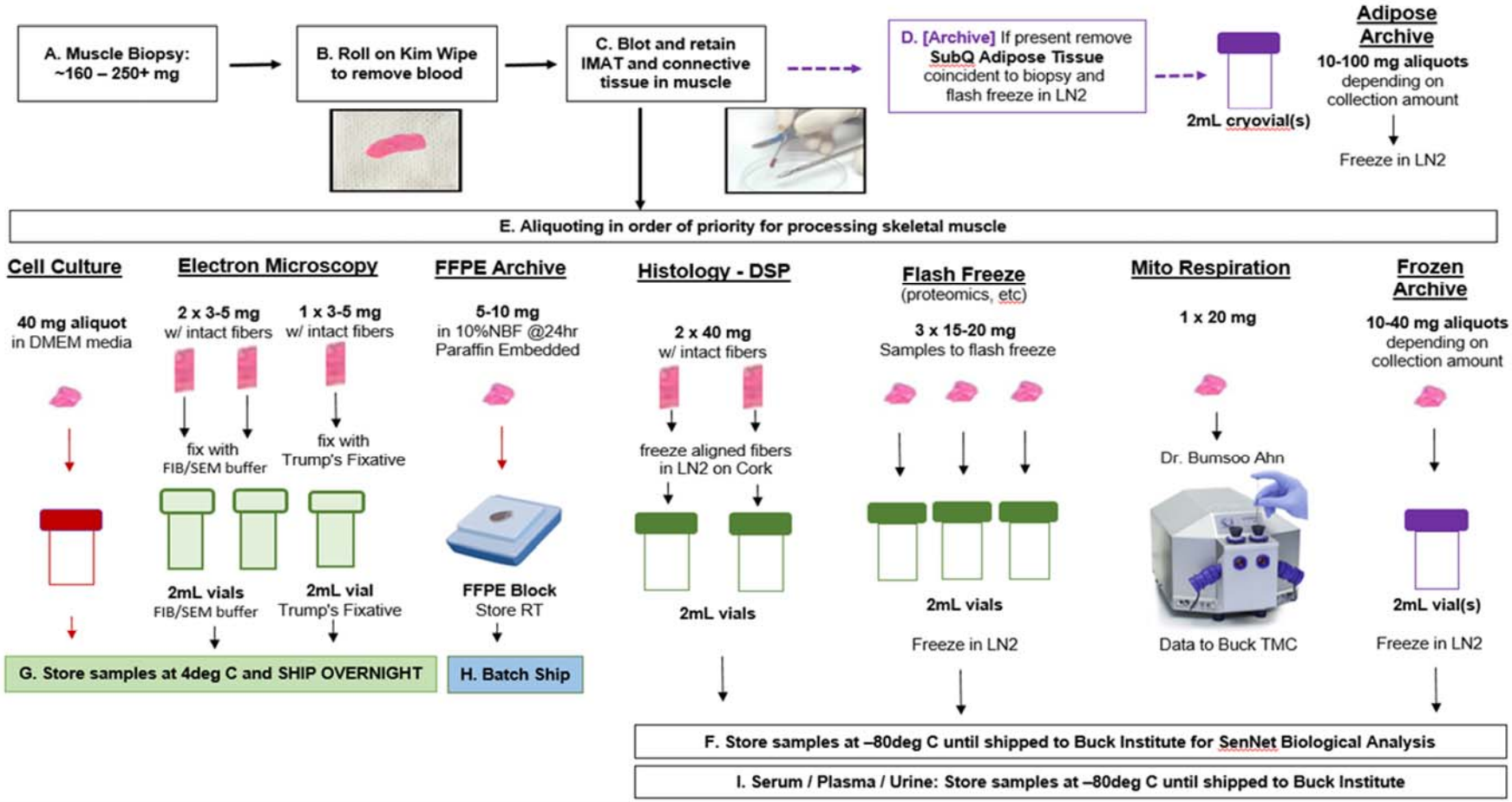
1. **[Frozen x2]** Remove a ~15-20mg piece of muscle and place into labeled cryovials and flash freeze in LN2. Store all samples flash frozen in LN2 at −80°C before shipping to the biorepository.

2. **[Mito Respiration]** Place ~20mg of tissue into 2.0 ml Biops buffer cryovial, keep on ice, process same day in Ahn lab.

3. **[Flash Freeze, Specimen Archive x2]** Any additional tissue remaining can be stored in any extra muscle tissue cryovials for archiving. Aliquot in to 15-20mg pieces depending on the sample size remaining (can fit up to 50mg per cryovial). Flash freeze in LN2

4. **[Flash Freeze, Adipose]** Retain visible adipose tissue, place it in the pre-labeled "Adipose Tissue" 2.0mL cryovial and flash freeze in LN2.

Figure 9.A Skeletal Muscle Processing


A. Muscle Biopsy: ~160 – 250+ mg
B. Roll on Kim Wipe to remove blood
C. Blot and retain IMAT and connective tissue in muscle
D. [Archive] If present remove SubQ Adipose Tissue coincident to biopsy and flash freeze in LN2
2mL cryovial(s)
Adipose Archive
10-100 mg aliquots depending on collection amount
Freeze in LN2
E. Aliquoting in order of priority for processing skeletal muscle
Cell Culture
40 mg aliquot in DMEM media
Electron Microscopy
2 x 3-5 mg w/ intact fibers
1 x 3-5 mg w/ intact fibers
fix with FIB/SEM buffer
fix with Trump's Fixative
2mL vials FIB/SEM buffer
2mL vial Trump's Fixative
FFPE Archive
5-10 mg in 10%NBF @24hr Paraffin Embedded
FFPE Block Store RT
Histology - DSP
2 x 40 mg w/ intact fibers
freeze aligned fibers in LN2 on Cork
2mL vials
Flash Freeze (proteomics, etc)
3 x 15-20 mg Samples to flash freeze
2mL vials
Freeze in LN2
Mito Respiration
1 x 20 mg
Dr. Bumsoo Ahn
Data to Buck TMC
Frozen Archive
10-40 mg aliquots depending on collection amount
2mL vial(s)
Freeze in LN2
G. Store samples at 4deg C and SHIP OVERNIGHT
H. Batch Ship
F. Store samples at –80deg C until shipped to Buck Institute for SenNet Biological Analysis
I. Serum / Plasma / Urine: Store samples at –80deg C until shipped to Buck Institute

10. DATA MANAGEMENT

Biospecimen data collection forms will be generated by Wake Forest Biogerontology Lab and align with data management systems as required by Buck SenNet TMC. All data collection forms will include de-identified unique participant tracking components, including acrostic, numerical ID, age, sex, and date of collections. Forms will also track specific biospecimen collections, time of collection, tissue weights, and processing checklists.

Clinical, health, and functional data will be input in REDCap data management systems at Wake Forest by study coordinators and data collectors. Biospecimen data is entered into data management systems as needed for SenNet TMC at Buck Institute. Local data management and entry related to Biospecimen will be overseen by site-PI Dr. Jamie Justice and Lab Supervisor, Heather Gregory.

11. SHIPPING

Samples collected are sent to the Buck Institute for processing (*except urine for D3-Creatine, which will be shipped to University of California in Berkeley, CA; see below)*. Samples will be stored onsite temporarily for batch shipping to the receiving site for Buck Institute SenNet TMC Biological Analysis Core.

Specimens are sent to the laboratory in batches when requested. Samples are shipped to Buck Institute using shipping labels provided by the Buck SenNet TMC.

Before shipping, an electronic manifest is created in the Biogerontology lab database. The electronic manifest should list at the top: “Buck Institute SenNet TMC Skeletal Muscle and Bio fluid Specimen” and Wake Forest and Biogerontology Lab supervisor, Heather Gregory’s name and contact information

For each specimen, the electronic manifest should list:
Cryovial, tube, or histology block ID
Date of specimen collection
On the day of shipping, email the following information:

- Electronic manifest
- The shipment tracking number (e.g. FedEx or UPS tracking number)
- Date of shipment
- expected arrival date
- Number of Styrofoam mailers shipped

Shipments to the laboratory are charged to local Federal Express or other account number.

This shipping protocol follows the procedures mandated by the International Air Transport Association’s Dangerous Goods Regulations-Packaging Instructions 650 and 904.
Samples should be prepared for shipping as follows in two participant box maps.

- 2 Participants per box for urine and blood samples
- 9 participants per box for the Muscle tissue samples

D3 specimens will be sent to the laboratory at UC Berkeley in batches of 50 samples.

Samples should be shipped to:

UNIVERSITY OF CALIFORNIA
HELLERSTEIN LAB / SHUBHA SHANKARAN
DEPT OF NUTRITIONAL SCI & TOXICOLOGY
54 MULFORD HALL
BERKELEY, CA 94720 USA
Lab Phone#: 510-642-0646
Cell Phone#: 925-286-1165

Before shipping, create an electronic manifest.
The electronic manifest should list at the top:
“SenNet Baseline urine specimen” and your clinic’s name and contact information

For each specimen, the electronic manifest should list:

- Tube ID (the SenNet ID)
- Date of specimen collection

The electronic manifest is just a simple list like shown below:

Participant ID#	Date of Collection
1000	5/27/2019
1001	5/28/2019
1002	5/28/2019
1003	5/29/2019
1004	6/01/2019
1005	6/05/2019

On the day of shipping, email shubha.shankaran@berkeley.edu the following information:

- Electronic manifest
- The shipment tracking number (e.g. FedEx or UPS tracking number)
- date of shipment
- expected arrival date
- number of styrofoam mailers shipped

A grid for inventory is not required.

Shipments to the laboratory are charged to your local Federal Express or other company’s account number.

This shipping protocol follows the procedures mandated by the International Air Transport Association’s Dangerous Goods Regulations-Packaging Instructions 650 and 904.

Samples should be prepared for shipping to the laboratory as follows:

- Wrap each freezer box in paper towels to absorb possible leakage. Put a rubber band around the towel-wrapped box or bag. Using two rubber bands, put a rubber band in each direction (horizontally and vertically), forming a cross with the rubber bands.
- Put the individual freezer boxes containing the samples into a leakproof zip-lock plastic bag and seal the bag.
- Place approximately one third of the dry ice on the bottom of the mailer.
- Carefully place the freezer box(es) into the styrofoam mailer. Place no more than a total

of 4 L of sample into the styrofoam shipping container. Although unlikely, use two or more styrofoam mailers for the shipment when necessary. (In this case, label the mailers “1 of 2” and “2 of 2”).

- Place the remaining dry ice (approximately 7 - 14 lbs. total) on top and around the samples to fill the styrofoam container.
- Enclose the styrofoam container in the outer cardboard box.
- Enclose the completed hard copy of the manifest
- Ensure that the date of the specimen collection is written on the cryovial

BOX#:_____ SPID 1291 SenNet BOX MAP

BARCODE LABEL HERE

URINE 1.5ML	URINE 1.5ML	URINE 1.5ML	URINE 1.5ML	URINE 1.5ML	URINE 1.5ML	URINE 1.5ML	URINE 1.5ML	PLASMA EDTA 0.5ML	PLASMA EDTA 0.5ML
PLASMA EDTA 0.5ML	PLASMA EDTA 0.5ML	PLASMA EDTA 0.5ML	PLASMA EDTA 0.5ML	PLASMA EDTA 0.5ML	PLASMA EDTA 0.5ML	PLASMA EDTA 0.5ML	PLASMA EDTA 0.5ML	PLASMA EDTA 0.5ML	PLASMA EDTA 0.5ML
PLASMA EDTA 0.5ML	PLASMA EDTA 0.5ML	PLASMA EDTA 0.5ML	PLASMA EDTA 0.5ML	PLASMA EDTA PLATELET POOR 0.5ML	PLASMA EDTA PLATELET POOR 0.5ML	PLASMA EDTA PLATELET POOR 0.5ML	PLASMA EDTA PLATELET POOR 0.5ML	PLASMA EDTA PLATELET POOR 0.5ML	PLASMA EDTA PLATELET POOR 0.5ML
PLASMA EDTA PLATELET POOR 0.5ML	PLASMA EDTA PLATELET POOR 0.5ML	SERUM 0.5ML	SERUM 0.5ML	SERUM 0.5ML	SERUM 0.5ML	SERUM 0.5ML	SERUM 0.5ML	SERUM 0.5ML	SERUM 0.5ML
				SERUM 0.5ML	SERUM 0.5ML	SERUM 0.5ML	SERUM 0.5ML	Empty	Empty

BARCODE LABEL HERE

URINE 1.5ML	URINE 1.5ML	URINE 1.5ML	URINE 1.5ML	URINE 1.5ML	URINE 1.5ML	URINE 1.5ML	URINE 1.5ML	PLASMA EDTA 0.5ML	PLASMA EDTA 0.5ML
PLASMA EDTA 0.5ML	PLASMA EDTA 0.5ML	PLASMA EDTA 0.5ML	PLASMA EDTA 0.5ML	PLASMA EDTA 0.5ML	PLASMA EDTA 0.5ML	PLASMA EDTA 0.5ML	PLASMA EDTA 0.5ML	PLASMA EDTA 0.5ML	PLASMA EDTA 0.5ML
PLASMA EDTA 0.5ML	PLASMA EDTA 0.5ML	PLASMA EDTA 0.5ML	PLASMA EDTA 0.5ML	PLASMA EDTA PLATELET POOR 0.5ML	PLASMA EDTA PLATELET POOR 0.5ML	PLASMA EDTA PLATELET POOR 0.5ML	PLASMA EDTA PLATELET POOR 0.5ML	PLASMA EDTA PLATELET POOR 0.5ML	PLASMA EDTA PLATELET POOR 0.5ML
PLASMA EDTA PLATELET POOR 0.5ML	PLASMA EDTA PLATELET POOR 0.5ML	SERUM 0.5ML	SERUM 0.5ML	SERUM 0.5ML	SERUM 0.5ML	SERUM 0.5ML	SERUM 0.5ML	SERUM 0.5ML	SERUM 0.5ML
SERUM 0.5ML	SERUM 0.5ML	SERUM 0.5ML	SERUM 0.5ML	SERUM 0.5ML	SERUM 0.5ML	SERUM 0.5ML	SERUM 0.5ML	Empty	Empty

SPID 1291 SenNet Muscle Collection BOX MAP XX=empty cell

BOX#:_____________

BARCODE LABEL HERE Histo-DSP 1	Histo-DSP-2	Histo-DSP-3	Frozen-1	Frozen 2	Frozen Arch 1	Frozen Arch 2	Adipose	xx
BARCODE LABEL HERE Histo-DSP 1	Histo-DSP-2	Histo-DSP-3	Frozen-1	Frozen 2	Frozen Arch 1	Frozen Arch 2	Adipose	xx
BARCODE LABEL HERE Histo-DSP 1	Histo-DSP-2	Histo-DSP-3	Frozen-1	Frozen 2	Frozen Arch 1	Frozen Arch 2	Adipose	xx
BARCODE LABEL HERE Histo-DSP 1	Histo-DSP-2	Histo-DSP-3	Frozen-1	Frozen 2	Frozen Arch 1	Frozen Arch 2	Adipose	xx
BARCODE LABEL HERE Histo-DSP 1	Histo-DSP-2	Histo-DSP-3	Frozen-1	Frozen 2	Frozen Arch 1	Frozen Arch 2	Adipose	xx
BARCODE LABEL HERE Histo-DSP 1	Histo-DSP-2	Histo-DSP-3	Frozen-1	Frozen 2	Frozen Arch 1	Frozen Arch 2	Adipose	xx
BARCODE LABEL HERE Histo-DSP 1	Histo-DSP-2	Histo-DSP-3	Frozen-1	Frozen 2	Frozen Arch 1	Frozen Arch 2	Adipose	xx
BARCODE LABEL HERE Histo-DSP 1	Histo-DSP-2	Histo-DSP-3	Frozen-1	Frozen 2	Frozen Arch 1	Frozen Arch 2	Adipose	xx
BARCODE LABEL HERE Histo-DSP-1	Histo-DSP-2	Histo-DSP-3	Frozen-1	Frozen 2	Frozen Arch 1	Frozen Arch 2	Adipose	xx