Standard Operating Procedures for Fluid Biomarker – Blood and Saliva Acquisition, Processing, Storage, Shipping

Accelerating Medicines Partnership® SCHIZOPHRENIA

An observational study examining clinical trajectories and predictors of outcomes in the clinical high risk population.

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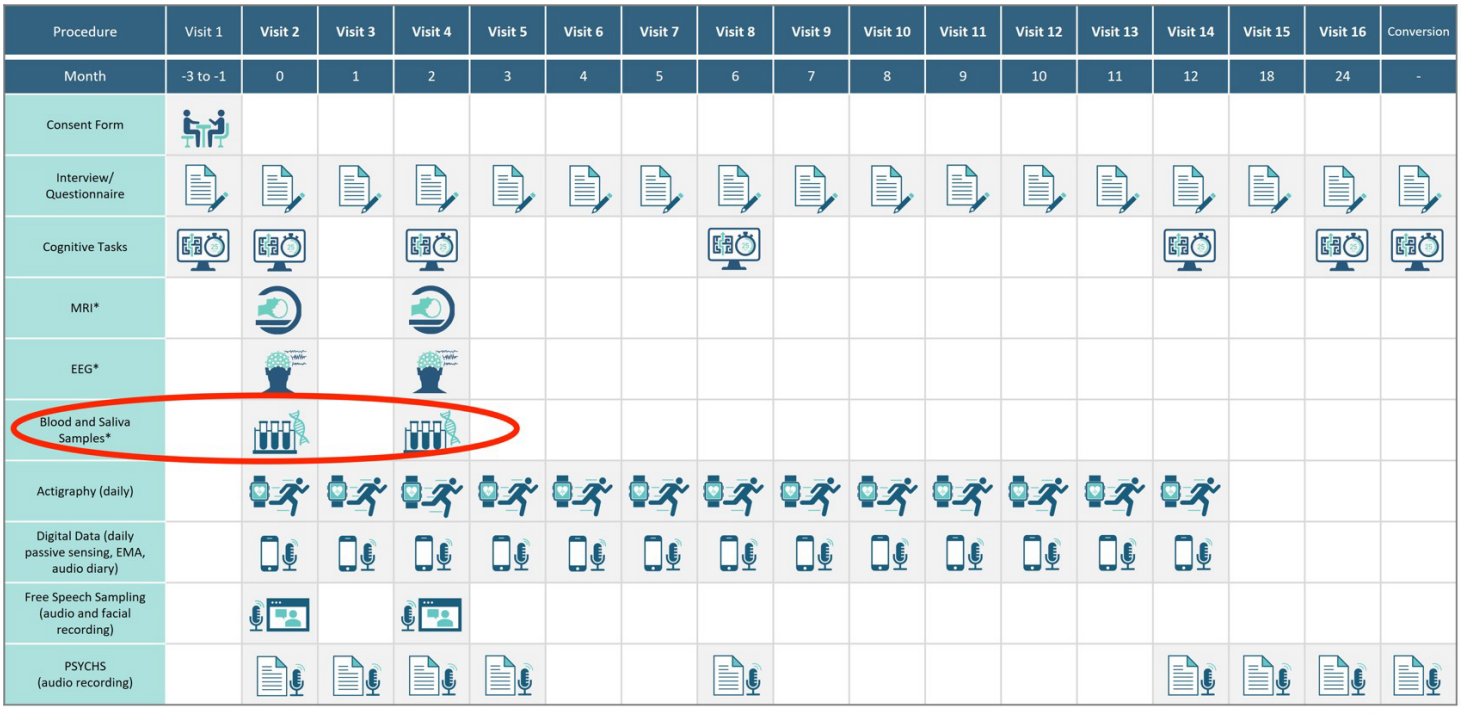


* In-person visit

Table of Contents

OVERVIEW: BLOOD SAMPLE PROCEDURES

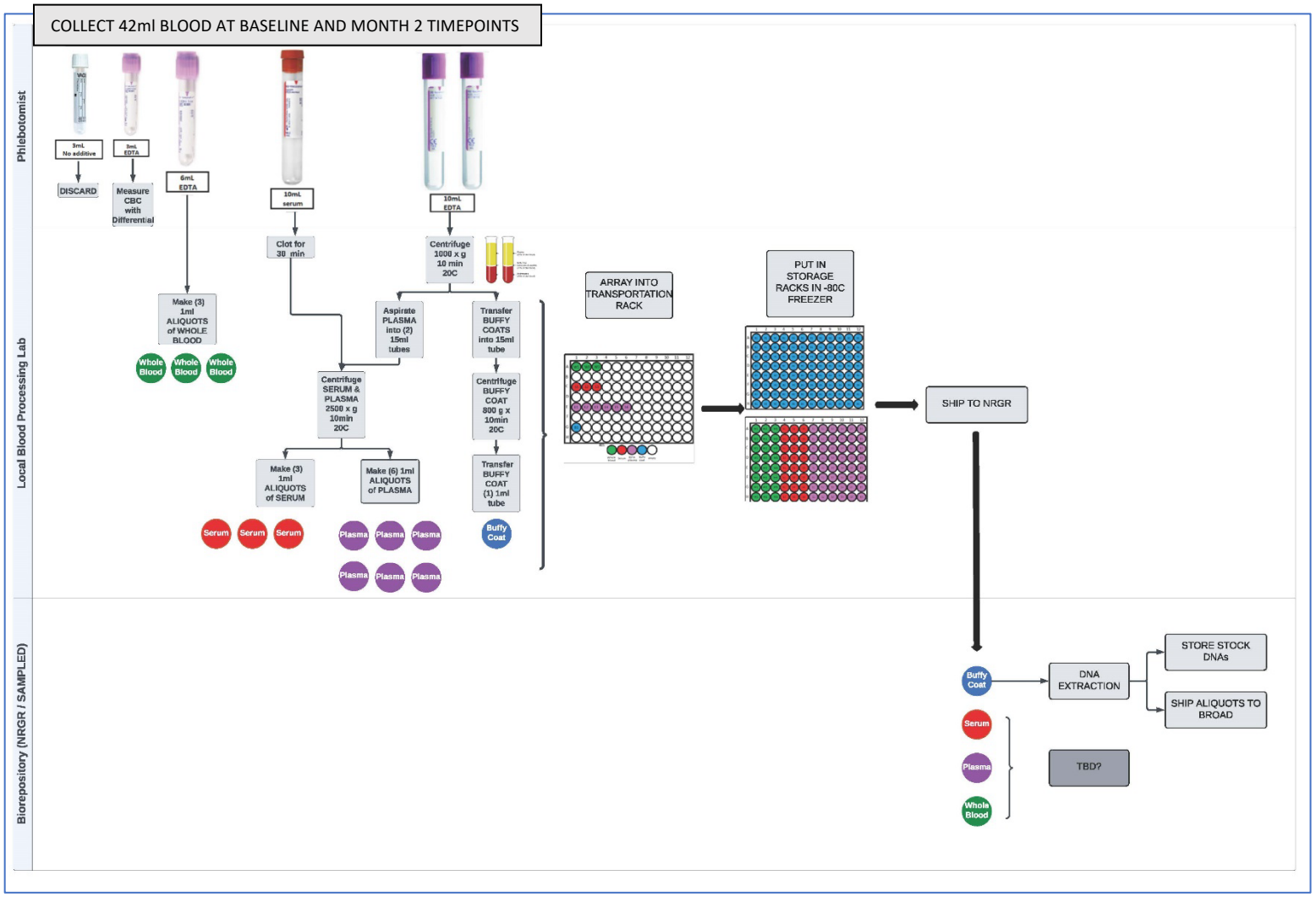
Study Research Coordinators are responsible for:

- **Providing study participants with written instructions** regarding the blood draw one week prior to study visit and reminding the study participants about these instructions the day prior to the blood draw.
- Completing the Current Health Status form (including collection of **Vital Signs**) prior to the blood draw.
- **Coordinating the blood draw** with the phlebotomist and **providing the phlebotomist with a copy of the study** Phlebotomy Guidelines **(**. Blood samples should be collected as close to noon as possible. Record the time of the blood draw and whether there were any problems with blood draw procedures on the Blood Sample Preanalytic Quality Assurance form.
- **Coordinating blood sample processing** with your blood processing laboratory facility **and providing the lab staff with the** Blood Sample Processing Protocols **and kit materials**. Samples should be processed and in the freezer within 90 minutes of collection, so coordination with the processing lab is important. Blood sample cryovials should be filled no more than 1mL. Overfilling cryovials can lead to issues with the rubber cap seal after freezing.
- Ensuring that the Blood Sample Preanalytic Quality Assurance data collection form is completed. **The study Research Coordinator needs to determine whether the study Research Coordinator or Blood Processing Facility Staff is responsible for scanning the cryotube and cryobox barcodes and recording the sample information (volume, position, time to freezing, etc.) into the study data base**. Obtain Vital Signs and Current Health Status prior to collecting blood samples. Record the time the samples arrived at the lab on the Blood Sample Preanalytic Quality Assurance form. Lab technicians will need to complete the rest of this form.
- Obtaining and preparing all blood collection, processing, and storage materials (see Supply List).
- Ensuring that all related data collection forms are completed in full and entered into the study database.

Data Collection Forms
Current Health Status
Blood Sample Preanalytic Quality Assurance
Complete Blood Count (CBC) with Differential
Daily Activity and Saliva Sample Collection

Overview: AMP-SCZ Blood Collection & Processing Workflow

Image created and provided by Felecia Cerrato, Associate Director for Neurobiology Projects at the Broad Institute.



PRE-STUDY VISIT INSTRUCTIONS FOR STUDY PARTICIPANTS: BLOOD SAMPLES

Blood Collection: Please provide study participants with the following information in writing at least one week prior to the study visit and again on the day before the study visit.

Instructions

You are scheduled for a blood draw on *(date scheduled)* at (*time scheduled*). In order for us to get accurate measurements from your blood, you are asked to follow some guidelines.

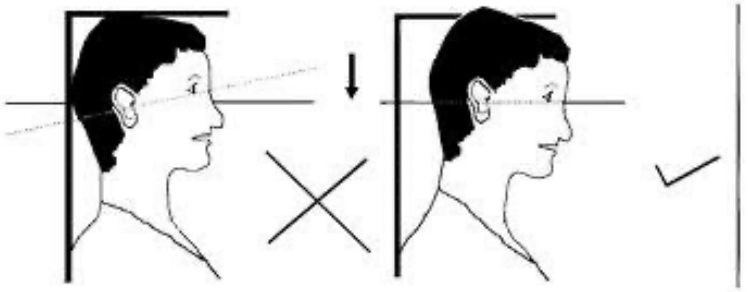
- Starting 2 days before your draw, please try to drink plenty of water. Hydration is key to an easy and quick blood draw for both you and your phlebotomist!
- Please try to not eat anything or drink anything other than water for **at least 4 hours** before your blood draw time – for this visit, you would stop eating and drinking (except water) (*time 4 hours prior to specimen collection**). This is because foods and drinks, including sugar, can impact the levels of some of the substances that will be measured.
- Please avoid using over-the-counter pain or cold medications **the week** before the study – for this visit, you would avoid using other-the-counter medications starting on (*day one week prior to specimen collection)*. This is because these medications may impact the levels of some of the substances that will be measured. Prescription medications should be taken according to the prescriber's instructions."
- Please be sure to get a good night's rest before your study visit.
- Please avoid rigorous exercise the day before or the day of your study visit.
- Please don't smoke, vape, or use any other tobacco or marijuana products at least two hours prior to your blood draw time.

**For example, if the blood is scheduled to be drawn at 12PM, the study participant should begin fasting at 8AM.*

CURRENT HEALTH STATUS FORM

The Current Health Status Form is completed immediately prior to or immediately after the blood draw.

A. Vital Signs

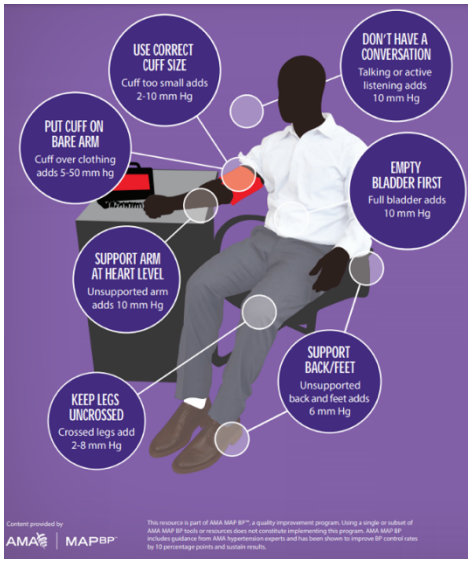


Height: Height is measured using a stadiometer.

- Ask the study participant to stand with back to wall, with shoulder blades and bottom touching wall.
- Ask the study participant to "stand up straight and look straight ahead".
- An imaginary line drawn from bottom of eye socket to top of ear canal should be parallel to floor. The stadiometer bar is placed on the top of the head, compressing hair as much as possible.
- Record the height, noting whether measurements are taken in inches or centimeters.

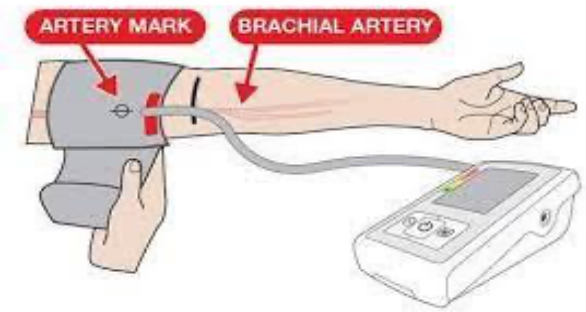
Weight: Weight is measured using a physician scale.

- Ask the study participant to remove shoes, jackets, sweaters, and belt.
- Have study participant empty his/her pockets.
- Have study participant stand on scale.
- Record weight, noting whether measurements are taken in pounds or kilograms.



Blood pressure and heart rate:

- Ensure that the blood pressure cuff is the correct size for the study participant's arm. You know you have the correct size for your study participant when the markings of the cuff intersect when wrapped around the study participant's upper arm.
- Have the study participant sit, with both feet flat on the ground (legs not crossed) and back supported.
- The upper arm should be bare.
- The arm should be supported so that the upper arm is at the level of the person's heart.
- Make sure the cuff is entirely deflated. Wrap the cuff around the arm, lining up the artery mark over the brachial artery as shown at right.
- Instruct the study participant to sit quietly while their blood pressure is being taken. Start the blood pressure machine.
- Don't have a conversation with the study participant while the blood pressure measurement is taking place!
- If the blood pressure is elevated (systolic greater than 130 and/or diastolic > 80), check to make sure the protocol is being followed, and re-check the blood pressure after the person has had a few minutes to relax.
- Record the blood pressure (higher number = systolic, lower number = diastolic) and heart rate.



- If the blood pressure is above 120/80 then retake the blood pressure after the study participant has sat quietly in the chair for several minutes.

- If two measurements are taken enter the second measurement.
- **Note about Good Clinical Practice:** Elevated blood pressure (hypertension) is defined as a **resting** systolic blood pressure of 140 or greater and/or a resting diastolic blood pressure of 90 or greater. However, it is **normal for a non-resting (such as when a person is active) blood pressure to be somewhat greater than 140/90**.
- Body Temperature: Temperatures are taken with a non-contact infrared thermometer.
- Check to make sure the device is operational (change batteries if needed).
- Make sure the study participant's wrist is bare.
- Hold the device about 4 inches (10 cm) above the study participant's wrist. Take the study participants temperature.
- Record the temperature, making sure to indicate whether the units are Fahrenheit or Centigrade.
- **Note about Good Clinical Practice:** If the body temperature is greater than or equal to 100.4°F (38°C), this indicates a fever. if it is hot outside or the person is overheated, the person's body temperature can be elevated, so if you do obtain a body temperature measurement this high, double-check that you are using the thermometer correctly and repeat the measurement. If necessary, have the participant sit quietly and try to cool down for a few minutes before re-measuring the body temperature. If the body temperature remains elevated, be sure to follow any procedures your institution has in place in case of fever.

B. Good Clinical Practice

The PI or someone designated by the PI at your site will receive a report of the blood pressure and body temperature measurements for abnormal values at each visit. For any measurements out of range, the PI or person designated by the PI will indicate whether each value is clinically significant or not clinically significant using the CBC with Differential Clinical Review Form and will record actions that were taken.

C. Activity and Inflammatory Conditions

Remind the study participant that, "It is important to be honest; that we understand sometimes a person can't avoid taking certain medications or eating or drinking, or just forgets about avoiding certain medications or fasting. It is much better for the study to know about these things; so we understand."

All dates are recorded using a 24-hour clock (e.g. 11am = 11:00, 11pm = 23:00, 12am = 00:00, 12pm = 12:00).

Instructions for recording sleep and wake times, occurrence of last meal and/or beverage, tobacco and/or marijuana use, and menstrual period.

- Record the date and time the study participant went to bed, woke up, last ate or drank, last used tobacco, last vaped and last time used marijuana using a 24-hour clock. For example, if the study participant went to bed at 11 pm and the study visit date is 11/05/2021, then the date for "went to bed" is 11/04/2021 (the day before the study visit date) and the time is 23:00. If the study participant went to bed at 1am, then the date for "went to bed" is 11/05/2021 (the same as the study visit date) and the time is 1:00.
- For menstruating study participants, ask about the date that their last menstrual period began and record.

Instructions for recording any inflammatory condition.

- Illness: Ask the study participant if they have been ill in the past month. If ill in the past month, ask the study participant when they last had any symptoms, and record the date. If they are having symptoms on the study day, then the date is the study visit date.
- Acne: Acne is rated by your observations using the Investigator Global Assessment of Acne (IGA) Rating Scale (scale provided on next pages).
  Sunburn: For rating recent sunburn, use the "Rule of Nines" sheet to calculate the percent body area involved (scale provided on next pages).

Instructions for recording physical activity.

- Ask the study participant: "How many minutes have you spent since you woke-up doing physical activity that was intense enough to make you breathe a little bit hard or work up a sweat?

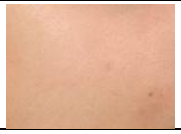
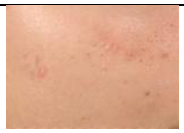
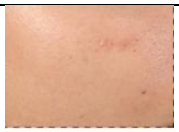
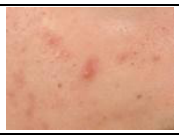
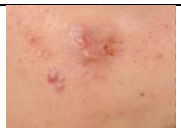
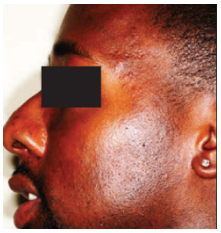
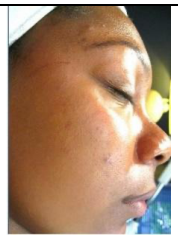
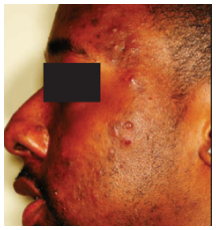
Instructions for reviewing the Medication Log.

- Obtain the study participant's medication log and review their current medications with them. Make updates as needed. Ask the study participant if they have begun taking any new medications. Make sure to ask specifically about (OTC or prescription) anti-inflammatories, pain medicine, allergy and/or cold medicine, diet pills, vitamins or other supplements, and antibiotics or steroids and to record when any of these medications was last taken.

Instructions for reviewing COVID-19 Infection and Vaccination.

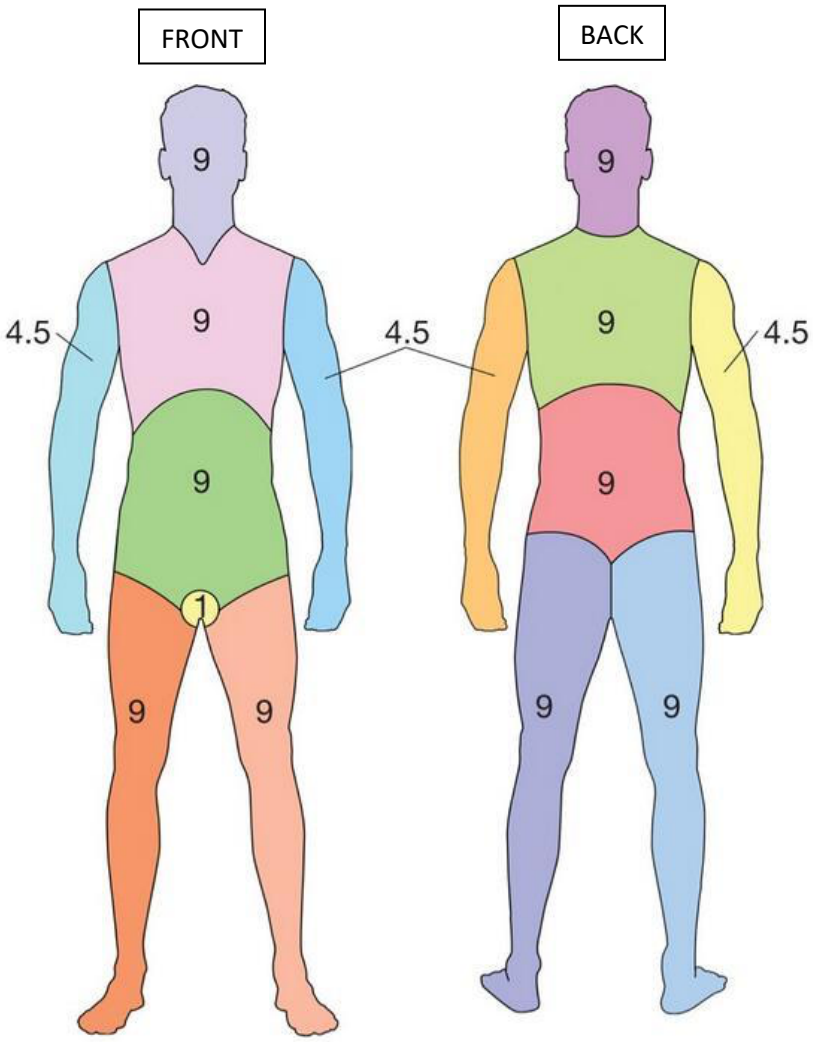
- Self-Explanatory.

D. Investigator Global Assessment of Acne (IGA) Rating Scale

Investigator Global Assessment of Acne (IGA) Rating Scale		
Grade 0 = 0	Grade 1 or Grade 2 = 1	Grade 3 or Grade 4 = 2
Clear. Residual hyperpigmentation and erythema (redness) may be present.	**Almost Clear**: A few scattered blackheads or whiteheads and a few small papules. **Mild**-Easily recognizable, less than half of the face is involved. Some blackheads or whiteheads and some papules and pustules.	**Moderate**: More than half of the face is involved. Many blackheads or whiteheads, papules and pustules. One nodule may be present. **Severe**: Entire face is involved, covered with blackheads and whiteheads, numerous papules and pustules, and a few nodules and cysts.
	 	 
	 	 
		 

E. Rule of Nines Sunburn Rating Scale

Instructions: Ask study participant if they have a sunburn. If yes, ask the study participant to indicate what body areas are involved. Rate the percent of the body involved using the following scale. For example, if a study participant's arms and lower legs were sunburned in the back but not the front, the score would be 4.5+4.5+9+9=27.



BLOOD SAMPLE PREANALYTIC QUALITY ASSURANCE FORM

This form is completed with information obtained during the blood draw and during and after blood processing.

- Label the blood tubes according to your phlebotomy lab requirements.
- Provide the phlebotomist with the Phlebotomy Guidelines at each blood draw. After the blood draw ask the phlebotomist if there were any deviations from the guidelines and if so, record these on this form.
- Send the 3mL EDTA tube (smallest purple top tube) to the local hematology lab for complete blood count (CBC) testing with differential following your site-specific protocol.
- Bring the remaining collected blood samples to the blood processing facility for processing and storage.
- Provide the blood storage cryovials to the blood processing facility.
- Follow your site-specific protocol regarding:
    - Recording date and time processing lab received blood samples.
    - Recording the temperature of the centrifuge and temperature units.
    - Scanning cryovial and cryovial storage rack barcodes into the study database. Remember, either the person performing blood processing or the study Research Coordinator must scan the cyrovial barcodes into the study database, as this is the only means of linking the stored sample to the study participant ID.
    - The cryovials used for whole blood, serum, plasma, and buffy coat storage are pre-barcoded with 2D barcodes on the bottom of each vial. The barcode must be scanned into the study database with a barcode reader. The barcode cannot be entered manually. Once the barcodes are entered into the database, sites should ensure that sample types (e.g. serum, plasma) and aliquot numbers (S1, S2, etc.) do not become mixed up.
    - Recording aliquot volumes.
    - Rating of serum and plasma specimen hemolysis according to study scale.
    - Rating presence/absence of lipemia according to study scale.
    - Recording of storage position of each cryovial, following the study protocol
    - Recording the cryovial storage rack description and the freezer ID in which samples are stored.
    - Recording any problems encountered during blood processing (refer to Blood Sample Processing Procedures, section D, step 9 on page 24).

A. Supplemental Blood Sample Preanalytic Quality Assurance Form

- The Supplemental Blood Sample Preanalytic Quality Assurance Form is an optional form available to sites whose blood processing facilities do not have direct data entry capabilities. This form allows some of the variables collected on the Blood Sample Preanalytic Quality Assurance Form to be collected on paper rather than via direct data entry; however, variables not collected on the Blood Sample Preanalytic Quality Assurance Form (e.g. the blood sample cryovial barcodes) must still be collected at the study visit.
- If sites plan to use this form, study staff must prepare the sample aliquot tubes (scan barcodes into database, hand-label and cap tubes, etc.) according to study standard operating procedures PRIOR TO providing the blood samples and sample aliquot tubes to the blood processing facility.
- Study staff must also ensure that they obtain this completed form from the blood processing facility staff after all samples have been processed and stored.
- **It is ultimately the study staff's responsibility to ensure that all blood samples are collected, processed, and stored correctly and that all required data is collected and entered into the study database in a timely manner. Study staff are responsible for storing any paper forms for site monitoring compliance.**

BLOOD SAMPLE COLLECTION PROCEDURES

A. Summary of Collection Procedures

- *Data for the Blood Sample Preanalytic Quality Assurance data collection form is collected before and during biospecimen collection and processing.* You will need to coordinate data collection with your phlebotomist and your blood processing laboratory. Please ensure that the required data is recorded during the collection and processing procedures.
- Phlebotomy guidelines are provided here. *Please provide phlebotomy guidelines to the phlebotomist at every blood draw.*
- The phlebotomist is required to provide the time of the blood draw and indicate whether there were any collection protocol deviations or difficulties. This information is recorded on the Blood Sample Preanalytic Quality Assurance form.
- The blood samples must be brought to the processing facility and processed **immediately** after collection. See section on Blood Sample Processing Procedures for details.
- Once collected, ensure that the blood tubes remain upright at all times. Always place tubes in a sample rack, not on their sides. Be careful to maintain an upright placement while samples are being transported to the lab and avoid shaking or jostling the samples during transport.
- Once collected, *ensure that the blood tubes remain at approximately 20°C (e.g., room temperature) at all times*. Blood is alive; extreme cold or heat will activate and/or damage blood cells. If transporting blood to your processing lab requires you to be outside in cold or heat, place tubes in a temperature-controlled container during transport.

B. Visit Preparation: Preparing the Study participant

- Ask the study participant about their previous experiences with having their blood drawn.
- If the study participant expresses anxiety around the blood draw, a fear of needles, or some other concerns, take steps to address these concerns:
    - If the subject is concerned about risks of a blood draw inform the study participant that:
        - the amount of blood that is being collected is small, about 3 tablespoons. This volume is not enough to affect the study participant's body.
        - the main risks from a blood draw are bruising at the site of the blood draw, that the needle might hurt, and that some people may become light-headed or faint.
    - If the study participant has a history of feeling light-headed or fainting, or is concerned about this risk:
        - Offer the subject either a sitting or lying down position
        - Inform the study participant that feeling light-headed, or fainting is a reflex response (the vasovagal reflex, or vasovagal syncope). Feeling light-headed or fainting is not due to the amount of blood that is taken.
        - Explain and have the study participant practice the Applied Tension Technique to study participant as a tool to prevent feeling light-headed or fainting.
        - Inform the phlebotomist about the history of feeling light-headed/fainting.
    - If the study participant expresses anxiety or distress about the blood draw:
        - Offer to distract the study participant during the blood draw by engaging in conversation.
        - Offer to help the study participant discuss any of their concerns with the phlebotomist.

The Applied Tension Technique to Prevent Vasovagal Syncope

1. Done while seated, a few minutes prior to blood draw, and during the blood draw.
2. Tense muscles in legs, arm, and upper body. Hold for 10-15 seconds, or until you feel warmth rising in your face. (Do not tense the arm that is used for the blood draw during the blood draw.)
3. Release tension for 20-30 seconds.
4. Repeat 5 times prior to the blood draw, and during the blood draw.

C. Visit Preparation: Blood Specimen Collection[1]

PRESCIENT

1. Coordinate blood draw date/time with phlebotomist and staff availability at your blood processing laboratory. Ensure that case(s) of empty Micronics cryovials (for storage of samples) and cap clusters (all colors listed on item 4 below) have been provided to the laboratory and blood processing facility in advance.
2. Assemble kit containing blood collection tubes (see item 3 below) and a copy of the Blood Sample Preanalytic Quality Assurance Form. Document subject ID, visit type/number (i.e., baseline/visit 2), sample collection date and time (following phlebotomy) on this form.
   .
3.

Quantity	Component
1	No-additive tube (3mL)
1	Red-top serum tube (10 mL)
1	Purple-top K2EDTA tube (3 mL)
1	Purple-top K2EDTA tube (5 mL)
2	Purple-top K2EDTA tubes (10 mL)

4. It is recommended that the blood processing technician prepares the cryovials accordingly during the processing procedure (if the blood processing technician is unable to prepare the cryovials accordingly, please contact the site Research Coordinator for further direction):
   i. Write "W1, W2, W3…" on the filled cryovials with a permanent marker and cap with green caps.
   ii. Write "S1, S2, S3…" on the filled cryovials with a permanent marker and cap with red caps.

ProNET

1. Coordinate blood draw date/time with staff availability at your phlebotomy lab and blood processing facility.
2. The kits provided by the Genetics & Fluids Core and the required blood collection tubes (that should be given to the phlebotomist) and the required blood storage tubes (that will be given to the blood processing facility). Sites are responsible for providing their phlebotomy and blood processing labs with all needed materials, even those not included in the kits (see Supply List).
3. Obtain an unexpired biospecimen visit kit (same kit used for saliva collection if saliva has been collected).
4. Place the blood collection tubes from the kit in a plastic bag to provide to your phlebotomist. Label tubes according to your facility requirements.

Quantity	Kit Component
1	No-additive tube (3mL)
1	Red-top serum tube (10 mL)
1	Purple-top K2EDTA tube (3 mL)
1	Purple-top K2EDTA tube (5 mL)
2	Purple-top K2EDTA tubes (10 mL)

5. The cryovials are provided to the Blood Processing technician in the transportation rack (this rack is re-used for sample preparation and transportation at every visit).

Quantity	Kit Component
13	Cryovials for whole blood samples, serum samples, EDTA plasma samples, and buffy coat sample

6. Arrange the cryovials in the transportation rack, cap them (*using the caps that were provided along with your*

[1] PRESCIENT and ProNET data collection and data entry methodologies will slightly differ throughout the SOPs. These differences do not impact sample quality and thus may be tailored to the sample processing lab's routines.

iii. Write "E1, E2, E3, E4, E5, E6…" on the filled cryovials with a permanent marker and cap with purple caps.

iv. Write "B1" on one of the cryovials with a permanent marker and cap with a blue cap.

v. It is recommended that the blood processing technician scans the cryovial barcodes once the sample has been aliquoted into the cryovials (note that cryovial number can vary between bleeds). Scan the cryovial and the cryovial storage rack barcodes into the appropriate data entry fields in the RPMS (place cursor in data entry field and scan barcode on the bottom of the vial; scanned barcode should appear at the location of the cursor).

5. It is recommended that the Blood Processing Facility staff enters data from the completed Blood Sample Preanalytic Quality Assurance Form directly into the Research Project Management System (RPMS). The site Research Coordinator will arrange access to the RPMS for laboratory facility staff. Each laboratory facility will be given one username and password for access to the RPMS (https://data.orygen.org.au/)
6. The Blood Sample Preanalytic Quality Assurance Form (phlebotomy section completed) needs to be brought to the Blood Processing Laboratory along with the blood tubes after collection.

very first shipment of kits), and hand-label them according to the procedures described below. Note that the columns of the transportation rack are labelled with numbers 1-12 and the rows are labelled with letters A-H.

i. Write "W1, W2, and W3" on 3 of the cryovials with a permanent marker and cap with green caps. Place these vials in positions A1, A2, and A3 of the transportation rack.

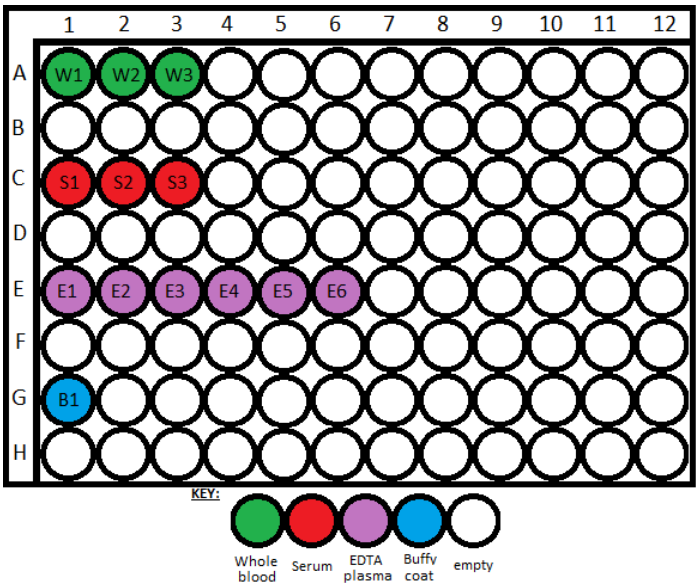
ii. Write "S1, S2, and S3" on 3 of the cryovials with a permanent marker and cap with red caps. Place these vials in positions C1, C2, and C3 of the transportation rack.

iii. Write "E1, E2, E3, E4, E5, and E6" on 3 of the cryovials with a permanent marker and cap with purple caps. Place these vials in positions E1, E2, E3, E4, E5, and E6 of the transportation rack.

iv. Write "B1" on one of the cryovials with a permanent marker and cap with a blue cap. Place this vial in position G1 of the transportation rack.

v. If your site-specific protocol specifies that the Research Coordinator scans the cryovial barcodes, then you will do so at this time. Scan the cryovial barcodes into the appropriate data entry fields in study database (place cursor in data entry field and scan barcode on bottom of vial; scanned barcode should appear at the location of the cursor).

7. Once all vials are prepared, the transportation rack should appear as shown **below**. To minimize the risk of sample mix-ups, it is very important that the cryovials are placed in exactly this way.



8. If the Blood Processing Facility staff is not entering data directly into the REDCap database, obtain a copy of the Supplemental Blood Sample Preanalytic Quality Assurance Form and write the study participant ID and sample date on the form.
9. The prepared cryovials in the transportation rack and the Supplemental Blood Sample Preanalytic Quality Assurance Form need to be brought to the Blood Processing Laboratory along with the blood tubes after collection. Study staff will need to ensure that they obtain the completed Supplemental Blood Sample Preanalytic Quality Assurance Form and now-empty transportation rack from the blood processing facility staff after all samples have been processed and stored correctly.

D. Phlebotomy Guidelines

Collection Device	Processing Facility	Baseline	2 Months
1 x No-additive tube (3mL)	Discarded after use	✓	✓
1 x Red-top serum tube (10 mL)	Sent to Blood Processing Laboratory	✓	✓
1 x Purple-top K2EDTA tube (3 mL)	Sent to Hematology Laboratory for a Complete Blood Count with Differential	✓	✓
1 x Purple-top K2EDTA tube (6 mL)	Sent to Blood Processing Laboratory	✓	✓
2 x Purple-top K2EDTA tubes (10 mL)	Sent to Blood Processing Laboratory	✓	✓

Instructions: It is important that the blood is handled gently, avoiding agitation, to minimize activation of blood cells and hemolysis. Either the phlebotomist or the study coordinator should document deviations from the recommended blood collection protocol on the Blood Sample Preanalytic Quality Assurance data collection form.

1. Place the blood collection tubes upright in a rack.
2. Record the time blood is drawn on **Blood Sample Preanalytic Quality Assurance** form.
3. Use a 21 gauge or larger butterfly needle. *Use of a smaller needle can result in hemolysis and activation of blood cells.*
4. An antecubital vein is highly preferred. Blood drawn from other sites will not accurately reflect circulating blood analyte levels. Apply the tourniquet 3-4 inches from the blood draw area. *Do not have study participant make a fist.*
5. *The tourniquet should be in place LESS than 60 seconds.* Longer duration will cause hemostasis, and thus activation of blood cells with the release of cytokines and other factors, interfering with the assays. If it has taken longer than 30 seconds to locate the vein, release the tourniquet, and wait 2-3 minutes before re-applying the tourniquet.
6. Locate the vein, swab with alcohol, allow a few seconds for the alcohol to dry (*drawing blood while alcohol is still wet results in hemolysis*).
7. Place the needle in the vein.
8. **Follow correct order of draw:**
   a. No additive tube
   b. 10mL serum tube
   c. EDTA tubes (3mL, 6mL, 10mL, 10mL in any order)
9. Note: If there is a tube failure during the blood draw, the phlebotomist may substitute a similar tube from their own supply.
10. To avoid backflow, make sure to keep blood tubes at or below level of venipuncture site.
11. Remove the tourniquet and change tubes as soon as blood begins to enter the tube.
12. Ensure that blood has stopped flowing and that a tube is completely filled before switching tubes.
13. After the blood tubes are filled, gently **invert the EDTA tubes 8 times** and the **serum (red top) tube 5 times.** This ensures adequate mixing of blood with tube additives; for plasma, microclots are prevented, and for serum, the presence of fibrin is prevented.
14. Return blood tubes to rack upright. Do not lay tubes down on their sides.
15. After last tube is drawn remove needle, activate safety mechanism to cover needle, and place in sharps container. Apply pressure and raise arm so that puncture is above heart level. Bandage as needed.

BLOOD SAMPLE PROCESSING PROCEDURES

BLOOD SAMPLE PROCESSING FLOW CHART

all processing steps must be completed within 90 minutes of blood collection

NOTE: The appearance of the blood collection tubes (including the color of the top of the tube) may vary based on the brand of the tube. **Make sure to read the label of the tube to determine the tube type.**

3mL No additive

Discard after collection.

3mL EDTA

Send to lab for CBC with differential.

6mL EDTA

Transfer 1.0mL of whole blood into each of the three (3) barcoded whole blood aliquot cryovials (**green** caps).

Freeze at -80°C.

10mL serum

Allow to sit upright and clot for 30 minutes.

10mL EDTA

Centrifuge at ***1000 x g* for 10 minutes at 20°C with brake off.

Transfer the plasma layers into 15mL centrifuge tubes.

Centrifuge ***both*** the 10mL serum tube and the 15mL centrifuge tubes containing plasma at 2500 x g for 10 minutes at 20°C with brake off.

Transfer 1.0mL of serum into each of the three (3) barcoded serum aliquot tubes (**red** caps).

Freeze at -80°C.

Transfer 1.0mL of plasma into each of the six (6) barcoded plasma aliquot tubes (**purple** caps).

Freeze at -80°C.

Transfer the buffy coat layers from both of the 10mL EDTA plasma tubes into a single 15mL centrifuge tube.

Centrifuge the 15mL centrifuge tube containing buffy coat at 800 x g for 10 minutes at 20°C with brake off.

Transfer the buffy coat layer into the one (1) barcoded buffy coat aliquot cryovial (**blue** cap).

Freeze at -80°C.

Due to feedback, the first centrifugation of the two EDTA tubes has been edited from ***600 x g* to ***1000 x g*** by the ProNET team. However, this change is up to the discretion of the lab technician processing the blood.

A. General Guidelines

- The blood samples must be brought to the local hematology lab and blood processing facility and processed **immediately** after collection.
    - The **3mL No Additive tube** is discarded after collection.
    - The **3mL EDTA tube (purple top)** is brought to your local lab for a CBC with differential according to the site-specific procedures.
    - The **10mL serum tube (red top), 6mL EDTA tube (purple top), and two 10mL EDTA tubes (purple top)** should be taken to the lab and processed as soon as possible after collection to minimize degradation. **The time from the blood draw to the samples being frozen should be less than 90 minutes.**
- Blood tubes must be kept upright once collected. Always place tubes in a sample rack, not on their sides. Be careful to maintain an upright placement while samples are being transported to the lab and avoid shaking or jostling the samples during transport.
- Blood tubes must be kept at approximately 20°C (e.g., room temperature) once collected, and all processing steps are done at approximately 20°C, including centrifugation. Blood is alive; extreme cold or heat will activate and/or damage blood cells. If transporting blood to your processing lab requires you to be outside in cold or heat, place tubes in a temperature-controlled container during transport.
- A transfer pipette can be used instead of a single channel multivolume pipettor for all steps except those that involve transferring sample into barcoded sample cryovials. A single channel multivolume pipettor set to exactly 1mL (1000uL) must be used for steps involving the transfer of samples into barcoded sample cryovials to ensure that the cryovials are not overfilled.
- Provide your blood processing facility staff with the blood processing instructions.

B. Best Practices

All processing labs have their own guiding rules and practices, however there are practices that are standard across all lab settings. We understand that some staff may be processing biosamples for the first time, so this section is included for those unfamiliar with lab standards.

- Please use the resources below to learn about best pipetting practices.
    - A Guide to Proper Pipetting (Article)
    - Micropipette Basics (Video)
    - Common Pipetting Mistakes (Video)
- Between 250-1000 uL are pipetted into each cryovial. Overfilling, that is putting more than 1000 uL into the cryovial, may result in the lids "popping off" or the tube cracking. This is because water expands when it is frozen.
- Overfilled cryovials with loose caps need to be corrected. We ask you to inspect cryovials for loose caps before shipping specimens. Overfilled cryovials with loose caps need to be addressed prior to shipping.
- Tools used in the lab should be **properly calibrated** and certified by specialized technicians at least once per year. Many universities and hospitals have contracts for centrifuge calibration and multivolume pipette calibration, but please reach

Example of cryovial with 1 mL of serum.


Example of cryovial that has been overfilled with more than 1mL of serum. Note that the caps are no longer secure.

out to us if you need assistance finding resources in your area. Improperly calibrated pipettes can lead to overfilling!

C. Transfer of Samples to the Blood Processing Facility

- Blood Processing Facility staff will receive one 10mL serum tube, one 6mL EDTA tube, and two 10mL EDTA tubes immediately after they are collected.
- Blood Processing Facility staff will also need access to three (3) 15mL centrifuge tubes, a 1000uL multivolume pipettor, 1000uL pipette tips, and a manual cryovial decapper.

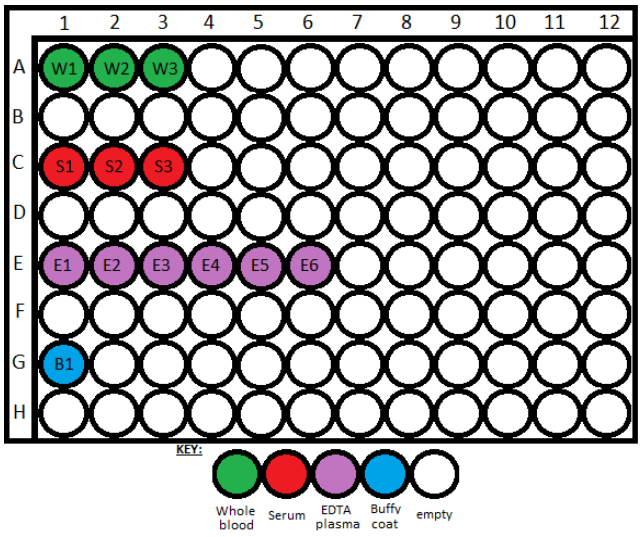
PRESCIENT

1. Blood processing staff will receive (in advance) case(s) of empty Micronics cryovials and cap clusters (see below): The green caps (to be placed on filled cryovials marked W1, W2, W3…) are for whole blood, the red caps (to be placed on filled cryovials marked S1, S2, S3…) are for serum, the purple caps (to be placed on filled cryovials marked E1, E2, E3, E4, E5, E6…) are for EDTA plasma, and the blue caps(to be placed on filled cryovial marked B1) is for the buffy coat sample.

   It is recommended for lab processing staff to store samples according to sample type and visit number. (e.g., serum tubes together, baseline samples together etc.).

ProNET

1. Blood processing staff will also receive a transportation rack containing 13 pre-scanned and capped cryovials in the following positions:



2. The green capped cryovials (marked W1, W2, and W3) are for whole blood, the red capped cryovials (marked S1, S2, and S2) are for serum, the purple capped cryovials (marked E1, E2, E3, E4, E5, and E6) are for EDTA plasma, and the blue capped cryovial (marked B1) is for the buffy coat sample.

D. Detailed Blood Processing and Storage Procedures

1. Record the time the biospecimens are received by the lab in the Blood Sample Preanalytic Quality Assurance data collection form.
2. Blood should be kept at ~20°C (room temperature) during processing. Make sure the centrifuge temperature is set to 20°C. Exposure to cold temperatures has been shown to activate platelets resulting in release of cytokines and other analytes of interest to our study, artificially elevating results.
3. Centrifugation speeds are given in g-force (same as RCF).
4. Centrifugation is always with the centrifuge brake is off.
5. When removing caps from capped cryovials with the supplied decapper, ensure that the caps are placed on a sterile surface and are not contaminated before recapping the cryovial.

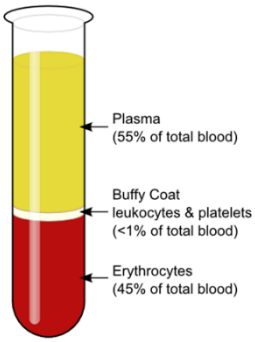
I. Processing the 10mL Serum tube, 6mL EDTA tube, and Two 10mL EDTA Tubes

1. Allow the 10mL serum tube to sit upright in a rack on the lab bench for **approximately 30 minutes** to clot.

2. First centrifugation of EDTA plasma:
    a. While the 10mL serum tube is clotting, centrifuge the two 10mL EDTA tubes at **1000 x g for 10 minutes at 20°C** with brake off.
        i. If the buffy coat is not clearly visible, re-centrifuge the EDTA tubes.
        ii. The ProNET and PRESCIENT teams edited this centrifugation speed edited from *600 x g* to *1000 x g*. However, this change is up to the discretion of the lab technician processing the blood. If your site has not had any issues with buffy coat extraction, continue with 600 x g.

3. Store aliquots of whole blood:
    a. While the plasma is undergoing the first centrifugation, prepare to aliquot the whole blood from the 6mL EDTA tube. A total of 3mL whole blood will be stored.
    b. Before uncapping the tube, gently invert the tube several times to ensure mixing of the contents.
    c. Use a single channel multivolume pipettor. Distribute the available whole blood between the three 1400uL cryovials (green cap), pipetting a minimum of 250uL and a maximum of 1000uL. If 3000uL of blood are available pipette 1000uL into each cryovial. **Take care to not pipette more than 1000uL as this can lead to cracked tubes or the lids popping off when frozen**. **A transfer pipet cannot be used for this step.**
        i. Replace the cap on the vials.
        ii. Record the aliquoted volumes.
            1. If the aliquoted volume is more than 1000uL, use a transfer pipet to remove fluid so the volume is no more than 1000uL. Use the picture on page 24 if you need a guide for volumes.

4. Transfer plasma from the 10mL EDTA tubes to 15mL centrifuge tubes:
    a. After centrifugation of the two 10mL EDTA tubes, carefully remove the tubes from the centrifuge and place them in a rack on the lab bench. Be careful not to disturb the blood. (*If the blood layers are disturbed the centrifugation must be repeated.)*
    b. Move two 15mL centrifuge tubes to the lab bench and label them with the subject ID and the date.
    c. Using a pipette, slowly aspirate the plasma layer of a 10mL EDTA tubes, being sure not to touch or disturb the red blood cells or buffy coat layer. Make sure to **leave approximately 500uL of plasma** in the blood collection tube to minimize risk of contamination by cells in the buffy coat. Transfer the plasma from one of the 10mL EDTA tubes into one of the 15mL centrifuge tube. Repeat these steps with the other 10mL EDTA tube and the other 15mL centrifuge tube. *(If the red blood cells or buffy coat layer are disturbed repeat the centrifugation step).*



    d. After aspirating the plasma layer and transferring it into a centrifuge tube, ***make sure to retain and leave undisturbed the buffy coat layer and red blood cells that remain in the 10mL EDTA tubes (used in Step 7)***.

5. Second centrifugation of EDTA plasma and first centrifugation of serum tube:
    a. At this point the 10mL serum tube should have been **clotting** for **approximately 30 minutes** and can be centrifuged. Centrifuge the 10mL serum tube from Step 2 and the two 15mL centrifuge

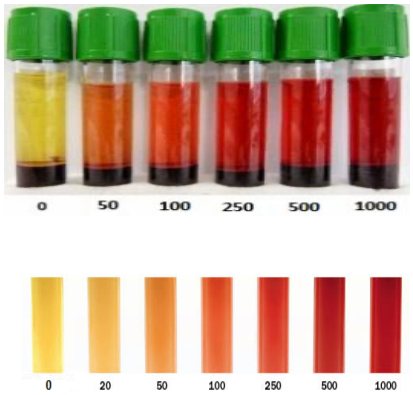
tubes containing the EDTA plasma at **2500 x g for 10 minutes at 20°C** with brake off. Counterweight appropriately.

b. Trouble-shooting: If fibrin strands are evident after centrifugation allow specimen to clot for an additional 30 minutes and then re-centrifuge.

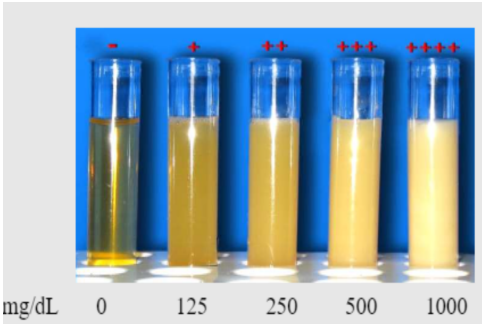
6. Harvest buffy coat:
   a. While the second centrifugation is under way, use a **sterile transfer pipette** to transfer the buffy coat layer from **each** of the two 10mL EDTA tubes into the single 15mL centrifuge tube.
      i. Place the tip of the transfer pipette just above the buffy coat layer.
      ii. Begin to draw the buffy coat layer into the transfer pipette. Move the transfer pipet in a spiral motion to draw the entire buffy coat layer into the pipette. Try to complete this step in a single motion.
   b. Set aside until centrifugation of the plasma and serum is complete. *(Try to minimize the amount of residual plasma and RBCs that are collected with the buffy coat.)*
7. Harvest platelet poor plasma and serum:
   a. After centrifugation of the 10mL serum tube and the two 15mL centrifuge tubes containing plasma from the two 10mL EDTA tubes, carefully remove the tubes from the centrifuge and place them in a rack on the lab bench.
   b. Begin centrifuging the 15mL centrifuge tube from Step 6 containing the buffy coat layer from the 2 10mL EDTA tubes at **800 x g for 10 minutes at 20°C** with brake off. Counterweight appropriately.
   c. Use a single channel multivolume pipettor. Distribute the available serum between the three 1400uL cryovials (red cap), pipetting a minimum of 250uL and a maximum of 1000uL. If 3000uL of serum are available pipette 1000uL into each cryovial. **Take care to not pipette more than 1000uL as this can lead to cracked tubes or the lids popping off when frozen. A transfer pipette cannot be used for this step.**
      i. Be careful not to touch or disturb the red blood cell layer in the 10mL serum tube.
      ii. A total of 3mL of serum will be stored.
      iii. Replace the caps on the vials.
      iv. Record the aliquoted volumes and the presence of hemolysis and lipemia (see step d).
         1. If the aliquoted volume is more than 1000uL, use a transfer pipet to remove fluid so the volume is no more than 1000uL. Use the picture on page 24 if you need a guide for volumes.
   d. Using a transfer pipette, transfer the plasma from **each** centrifuge tube into **two** clean 15mL centrifuge tubes (avoid touching the pellet). Mix gently using the transfer pipette to homogenize the plasma in each centrifuge tube.
   e. Use a single channel multivolume pipettor. Distribute the available plasma between the six 1400uL cryovials (purple cap), pipetting a minimum of 250uL and a maximum of 1000uL. If 6000uL of serum are available pipette 1000uL into each cryovial. Take care to not pipette more than 1000uL as this can lead to cracked tubes or the lids popping off when frozen **A transfer pipette cannot be used for this step.**
      i. Ideally fill the first plasma cryovials from one 15mL centrifuge tube followed by the remaining plasma cryovials from the other 15mL centrifuge tube.
      ii. Be careful not to touch or disturb the pellet of platelets in the 15mL centrifuge tube.
      iii. A total of 6mL of EDTA plasma will be stored.
      iv. Replace the caps on the vials.
      v. Record the aliquoted volumes and the presence of hemolysis and lipemia (see step d).
         1. If the aliquoted volume is more than 1000uL, use a transfer pipet to remove fluid so the volume is no more than 1000uL. Use the picture on page 24 if you need a guide for volumes.

f. Evaluate the serum and plasma aliquots for hemolysis (below - indicated by color of plasma) and lipemia (at right - indicated by turbidity or cloudiness of plasma) using the examples provided and record in the Blood Sample Preanalytic Quality Assurance data collection form. *Lipemia should be rare in this cohort.*
    i. Lipemia must be rated once for serum and once for plasma.
    ii. Hemolysis must be rated once for serum and once for each of the plasma aliquots.

Hemolysis



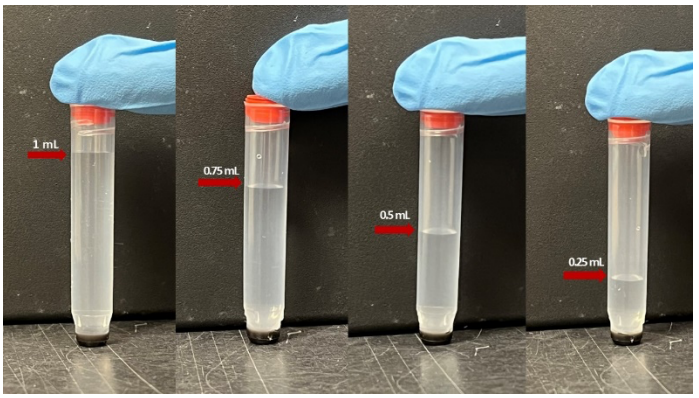
Lipemia



8. Centrifugation and storage of buffy coat:
    a. After centrifugation of the 15mL centrifuge tube containing the buffy coat layers from the 2 10mL EDTA tubes is complete, carefully remove the 15mL centrifuge tube from the centrifuge and place it in a rack on the lab bench.
    b. Use a **single channel multivolume pipettor** set to exactly 1mL (1000uL) with a clean tip and a gentle spiral motion to aspirate 1mL of buffy coat sample from the buffy coat layer in the 15mL centrifuge tube into the single 1.4mL buffy coat (blue cap) cryovial. **A transfer pipette can be used for this step to minimize aspiration of RBCs.**
        i. Try to minimize aspiration of residual RBCs that are layered under the buffy coat and residual plasma that is layered above the buffy coat.
        ii. A total of 1mL of buffy coat will be stored.
            1. Do not aliquot more than 1000uL of buffy coat into the cryovial. If the aliquoted volume is more than 1000uL, use a transfer pipet to remove fluid so the volume is no more than 1000uL. Use the picture below if you need a guide for volumes.
        iii. Replace the cap on the vial.
        iv. Record the aliquoted volume.

9. Storage of samples:

a. All samples must be stored at -80°C. **Double-check that you have correctly processed all samples** and recorded on Blood Sample Preanalytic Quality Assurance Form the aliquoted volume of each sample before you store the samples. It is extremely important to not overfill any cryovials, as this will lead to cap issues. **If any cryovials are filled above 1mL, please use a transfer pipette to remove some fluid so it is at or below 1mL.** Use the image to the right to help with volume estimation.



b. Place samples in a designated -80°C freezer according to the Blood Sample Storage Guidelines (below). When you pull the sample storage rack out of the freezer to store the newly collected samples in it, it is extremely important that samples already stored in the storage rack do not thaw. Do not take the storage rack out of the freezer for extended times. Take it out only momentarily to add the newly collected samples, and then place back in the freezer.
c. Once the samples are stored in the storage rack, record on Blood Sample Preanalytic Quality Assurance Form the storage position of each sample, the time the samples are stored at -80°C, the storage rack ID, and the freezer in which the samples are stored.
    i. Record any protocol deviations or issues with processing before saving this form. Some examples could be:
        1. "Buffy coat was not visible after first centrifugation of EDTA tubes. Lab tech decided to spin again at 800g for 10 minutes, with brake off."
        2. "One EDTA tube only filled halfway during blood draw. This resulted in 3 of the 6 plasma cryovials aliquoted with 0.5mL of plasma each."
        3. "Centrifugation malfunction caused delays with processing and therefore increased time to freezer to 100 minutes instead of 90 minutes."
d. After the samples are stored in the storage rack, the transportation rack must be returned to the study team.

E. Blood Sample Storage Guidelines

PRESCIENT

Sites are responsible for providing storage space and racks to store the samples following processing.

Please store each sample type for each visit together (e.g., serum tubes together, baseline samples together etc.). The specific cryovial storage rack description, the position, volume and freezing time of each sample must be recorded on the Blood Sample Preanalytic Quality Assurance Form and entered on the Research Project Management System (RPMS). Blood Processing Facility staff are required to scan the cryovial and cryovial storage rack barcodes and upload the completed Blood Sample Preanalytic Quality Assurance Form directly into the RPMS. A hard copy of this form should be stored in a

ProNET

Sample storage racks are provided by the Genetics & Fluids Core. The buffy coat samples will be stored separately from the whole blood, serum, and plasma samples. Each whole blood, serum, and plasma sample storage rack will be used to hold samples from **eight** study visits. Each buffy coat sample storage rack will be used to hold samples from **96** study visits. Samples will be stored as shown in the guide **below**.

The specific storage rack and position of each sample must be recorded on the Blood Sample Preanalytic Quality Assurance Form at time of collection and storage. This information will be used to ensure that the protocol is being followed appropriately and to assist sites with periodic sample

locked cabinet for collection by the research team. This information will be used to ensure that the protocol is being followed appropriately and to assist sites with periodically shipping samples to The Florey Institute in Melbourne, Australia.

Refer to sample position layouts below as a guide (row A to be filled first, from position 1 to 12). Do not leave spaces blank. Fill as many aliquots as available.

Baseline whole blood:

	1	2	3	4	5	6	7	8	9	10	11	12
A	W1	W2	W3	W4	W5	W6	W1	W2	W3	W4	W5	
B												
C												
D												
E												
F												
G												
H												

Month 2 Plasma

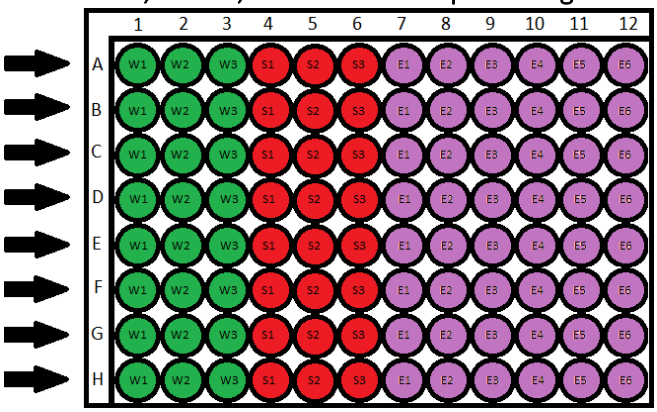
	1	2	3	4	5	6	7	8	9	10	11	12
A	E1	E2	E3	E4	E5	E6	E7	E1	E2	E3	E4	E5
B	E6											
C												
D												
E												
F												
G												
H												

Baseline Buffy Coat

	1	2	3	4	5	6	7	8	9	10	11	12
A	B1	B1	B1	B1	B1	B1	B1	B1	B1	B1	B1	
B												
C												
D												
E												
F												
G												

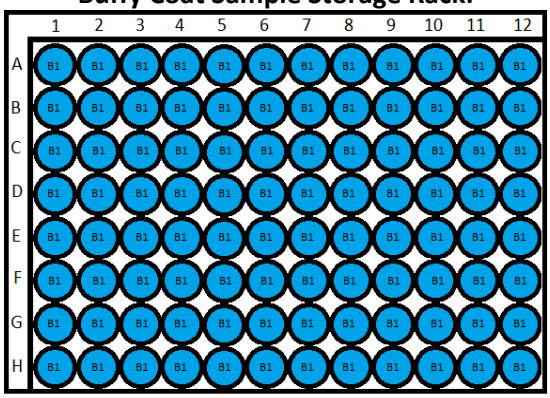
shipments. Samples should not be moved or rearranged once they are stored in the sample storage racks.

Whole Blood, Serum, and Plasma Sample Storage Racks:



Each of the 8 rows (A-H) holds whole blood, serum, and plasma samples from a single biomarker vsit. Samples must be stored in the order and positions shown. If one or more of the aliquots is not filled, the space that would have held that aliquot should be left empty and samples should continue to be stored in the format shown above. Once a sample storage rack holds samples from 8 biomarker visits, the next biomarker visit's samples should be stored in the next sample storage rack.

Buffy Coat Sample Storage Rack:



Buffy coat samples will be stored in a storage rack separate from the storage racks that hold whole blood, serum, and plasma samples. Each buffy coat sample storage rack will hold buffy coat samples from 96 biomarker visits.

H

COMPLETE BLOOD COUNT (CBC) WITH DIFFERENTIAL FORM

- CBC with differential results are to be entered into the study database (RPMS or REDCap) within 3 days of specimen collection.
- Sites must ensure that their results are entered using the measurement unit specified on the data entry form. Different labs have different conventions, especially when reporting cell counts. For example, some labs refer to a liter (L) of blood, others to a microliter (uL) of blood. The table, below, provides a few examples of how the same white blood cell count (WBC) might be reported, and how to enter the value into the study data base (the "x" means multiply by this number):

The result from your lab report:			How to enter in the study database:		
Test	**Result**	**Units**	**Test**	**Result**	**Units**
WBC	6.3	10^3/uL	WBC	6.3	10^9/L
WBC	6.3	10^3/uL	WBC	6.3	10^9/L
WBC	6.3	10^9/L	WBC	6.3	10^9/L
WBC	6300	cells/uL	WBC	6.3	10^9/L

- The laboratory report provides a reference range for normal values that is specific to that laboratory. Please familiarize yourself with how results that are outside of the reference range are reported.
- The cell types that are included in the WBC are neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Sometimes labs report these cell counts as a percent of the total number of white blood cells. The absolute number of the cell types, not the percentage, is entered on this form.
    - The sum of the absolute neutrophils, absolute lymphocytes, absolute monocytes, absolute eosinophils, and absolute basophils should approximately equal the white blood cell count. If this is not the case, the database will flag a possible error. Check to make sure you haven't misplaced a decimal point. This can happen, especially with the less common cell types. (For example, monocyte counts are typically less than 1 x 10^9/L. Be careful not to enter a value of 0.1 x10^9/L as 1.0 x 10^9/L).
- The CBC with differential form is considered a source document and should be uploaded to YaleBox to comply with GCP guidelines.

F. A. CBC with Differential: Good Clinical Practice

The PI or someone designated by the PI at your site will receive a notification of the CBC with Differential test results. If any values are out of range, the PI or person designated by the PI will indicate whether the value is "clinically significant". If the value is deemed clinically significant, the PI/designate will indicate actions taken. A copy of the CBC with Differential Review Form is provided in Appendix A.

OVERVIEW: SALIVA SAMPLE PROCEDURES

Research Coordinators are responsible for:

- **Providing study participants with written instructions** regarding saliva collection one week prior and reminding study participants about these instructions the day prior to the blood draw.
- Completing the Daily Activity and Saliva Sample Collection form during the saliva collection visit.
- Instructing study participants about how to collect their own saliva samples, including having study participants watch a brief instructional video, observing study participants while they provide saliva samples, and correcting any deviations from passive drool sample collection instructions.
- Ensuring that three saliva samples are collected over a two-hour period, as close to noon as possible (e.g. 1100, 1200, 1300). Saliva **collection takes from three to five minutes**, thus other study procedures are to be done in between. For example, during EEG visit: Sample #1 prior to EEG capping, Sample #2 an hour after Sample #1 (during a break period), Sample #3 two hours after Sample #1, after EEG is completed. It is preferred to collect saliva either on a different day from blood collection, or if that is not feasible prior to blood collection. While not preferred, it is allowed to collect saliva samples on the same day as but after blood is collected.
- Obtaining and preparing all saliva collection and storage materials (see Supply List).
- Ensuring that all related data collection forms are completed in full and entered into the study database.

Data Collection Forms
Daily Activity and Saliva Sample Collection

PRE-STUDY VISIT INSTRUCTIONS FOR STUDY PARTICIPANTS: SALIVA SAMPLES

Saliva Collection: Please provide study study participants with the following information in writing at least one week prior to the study visit and again on the day before the study visit.

Instructions

You are scheduled to have saliva samples collected on *(date scheduled).* In order to get accurate measurements from your saliva you are asked to follow some guidelines.

- The evening and morning before you come in for your appointment; please do not consume the following foods and beverages. Refrain from these foods and beverages starting at 6:00 p.m. (18:00 on the 24-hour clock) the night before giving saliva samples:
    - Foods and drinks that contain caffeine or xanthenes (e.g. chocolate, coca cola, tea, coffee, energy drinks, etc.)
    - Dairy products (e.g. milk, cheese, etc.)
    - Alcohol
- Please be sure to get a good night's rest before your study visit.
- Please avoid rigorous exercise the day before or the day of your study visit.
- Please don't smoke or vape at least two hours prior to your blood draw time.

DAILY ACTIVITY AND SALIVA SAMPLE COLLECTION FORM

A. Completion of Daily Activity section of the form

The majority of the Daily Activity and Saliva Sample Collection data collection form is completed just prior to the collection of saliva samples. The sample collection times, volumes, whether the study participant had a beverage within 10 minutes of each sample, and the storage location of each sample is collected as each saliva sample is collected. Please ensure that the required data is collected and recorded during the sample collection procedures.

Remind the study participant, "It is important to be honest; that we understand sometimes a person can't avoid certain activities or taking certain medications or eating or drinking, or just forgets about avoiding certain activity, medications or foods and drinks. It is much better for the study to know about these things; so, we understand."

- **Instructions for recording consumption of food and drinks.**
  Make sure to provide the study participant with examples of foods and drinks in each category. Ask the study participant whether he or she has recently **had a snack, meal, or beverage other than water, if he or she has brushed/flossed his or her teeth in the past 45 minutes**, or if she or he has had any **water to drink in the past 10 minutes.**
    a. If the study participant reports that he or she has **eaten or had a beverage other than water**, have the study participant **rinse his or her mouth with water and then wait 10 minutes** to collect the first sample.
    b. If the study participant reports that he or she has **brushed/flossed his or her teeth** in the past 45 minutes, it is **optimal to wait until 45 minutes** have passed since he or she brushed/flossed teeth to proceed with collection of samples. However, it is acceptable to proceed if needed due to scheduling constraints.
- **Instructions for reviewing the Medication Log.**
  Obtain the study participant's medication log and review their current medications (including last use) with them. Make updates as needed. Ask the study participant if they have begun taking any new medications. Make sure to ask specifically about (OTC or prescription) anti-inflammatories, pain medicine, allergy and/or cold medicine, diet pills, vitamins or other supplements, and antibiotics or steroids.
- **Instructions for reporting recent activity.**
  To estimate diurnal variation, we need to know the time the study participant went to bed and woke up and whether this time was on the same day as the study visit or the day before the study visit.
- **Saliva collection instructions for study participants.**
  Study participants collect their own saliva sample specimens, under your supervision. Begin by having the participant watch the 4-minute video explaining the procedure: https://salimetrics.com/passive-drool-saliva-collection-video/#header. Then orient the participants to collection tubes and other supplies.
- **Instructions for recording cryotube volumes**
  Study participants are instructed to **provide between 1.5mL and 1.8mL of saliva into each of the 2 cryovials**. If study participants are not able to provide this volume, estimate the volume that they were able to provide. Record the actual volume provided.
- **Instructions for recording saliva collection time**
  Record the time the study participant started the passive drool saliva collection procedure for both samples.
- **Instructions for recording Position in Cryotube Rack and Cryotube Rack Barcode and Description**
  Record this information per protocol.

SALIVA SAMPLE COLLECTION PROCEDURES

A. Visit Preparation: Saliva Sample Collection

PRESCIENT

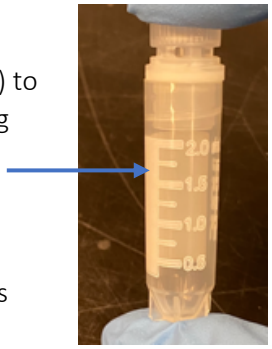
1. Assemble a saliva collection pack with 6 barcoded cryotubes and 3 Saliva Collection Aids..
2. Use a permanent marker to label the tubes "1A, 1B, 2A, 2B, 3A, and 3B."
3. Put ice in a small cooler marked with a biohazard label.
4. The cooler, prepared cryovials, and the Saliva Collection Aids are provided to the study staff member who is responsible for collecting the saliva samples.

ProNET

1. Obtain an unexpired biospecimen collection kit. Ensure that the same kit is used for saliva and blood collection.
2. Remove the six cryovials for saliva collection and the three Saliva Collection Aids from the kit. Use a permanent marker to label the tubes "1A, 1B, 2A, 2B, 3A, and 3B."
3. Scan the cryovial barcodes into the appropriate data entry fields in study database (place cursor in data entry field and scan barcode on bottom of vial; scanned barcode should appear at the location of the cursor).
4. Place the cryovials in the transportation rack.
5. Put ice in a small cooler marked with a biohazard label.
6. The cooler, the transportation rack of prepared cryovials, and the Saliva Collection Aids are provided to the study staff member who is responsible for collecting the saliva samples.

B. Detailed Saliva Collection and Storage Protocol

1. Collection of saliva samples:
    a. Collect the first saliva sample.
        i. Prior to sample collection study participants should watch the 4 minute instructional video available at the following link: https://salimetrics.com/passive-drool-saliva-collection-video/#header.
        ii. The ideal amount of saliva collected in each cryovial is 1.5mL. Request that participants try to get saliva (excluding any bubbles) to the 1.8mL line, to correct for any bubbles created while providing the sample. If the cryovial is overfilled, be sure to have a transfer pipet on hand to remove some saliva before freezing.



        iii. Study participants collect their own saliva samples. Provide the study participant with a saliva collection aid and the two cryovials labeled 1A and 1B. Double-check that you have provided the cryovials for the samples collected at the 1st timepoint, not at the 2nd or 3rd timepoint. Provide the study participant with a clean surface to use for saliva collection. Place the sample transportation rack in the cooler containing ice.

iv. Provide the study participant with a copy of the saliva collection instructions and review these instructions with the study participant:
    1. Open the foil pouch and remove the Saliva Collection Aid.
    2. Remove the cap from Cryotube 1A.
    3. Place one end of the Saliva Collection Aid into Cryotube 1A.
    4. Ask a participant to allow their saliva to pool in their mouth (for about 10 seconds).
v. Then, with head tilted forward, gently guide saliva through the Saliva Collection Aid into the vial. DO NOT SPIT/BLOW or otherwise force saliva into the tube, as this will create foaming and bubbles.
vi. After the vial is filled to the required volume, remove the Saliva Collection Aid and place the aid on the clean surface.
vii. Attach the cap to the collection vial and tighten completely.
viii. Once the vial is closed and the cap is secured, use a disinfectant wipe to wipe down the outside of the collection vial.
ix. Once the cryovial is wiped down, place it into the transportation rack on ice.
x. Repeat with Cryotube 1B using the same Saliva Collection Aid
xi. Once both samples are filled and stored on ice, discard the Saliva Collection Aid and disinfect the surface.

b. At the time that the first saliva collection ends, set timers for 60 minutes and 120 minutes.
c. Collect the second saliva sample.
    i. After 60 minutes, collect samples 2A and 2B as described above and then place on ice.
d. Collect the third saliva sample.
    i. After 120 minutes, collect samples 3A and 3B as described above and then place on ice.
e. The samples must remain on ice (refilling as necessary) until they are placed in the -20°C or below freezer. The samples must be placed in the -20°C or below freezer by the end of the day on the day of collection.

2. Store saliva samples:
    a. All samples must be stored at -20°C or below. Double-check that you have correctly recorded on Daily Activity and Saliva Sample Collection Form the volume and all other information required of each sample before you store the samples.
    b. Place samples in the -20°C or below freezer according to the Saliva Sample Storage Guidelines (below). When you pull the sample storage rack out of the freezer to store the newly collected samples in it, it is extremely important that samples already stored in the storage rack do not thaw. Do not take the storage rack out of the freezer for extended times. Take it out only momentarily to add the newly collected samples, and then place back in the freezer.
    c. Once the samples are stored in the storage rack, record on Daily Activity and Saliva Sample Collection Form the storage position of each sample, the time the samples are stored at -20°C or below, the storage rack ID, and the freezer in which the samples are stored.
    d. After the samples are stored in the storage rack, the transportation rack must be returned to the study team.

C. Saliva Sample Storage Guidelines

PRESCIENT

Sites are responsible for providing storage space and cryovial storage racks to laboratory facility staff in advance to store the samples following collection.

It is recommended for laboratory facility staff to store saliva samples according to visit number. (e.g., baseline samples together, month 2 samples together etc.).

The specific cryovial storage rack description, the position, volume and freezing time of each sample must be recorded on the Daily Activity and Saliva Sample Collection Form and entered directly on the Research Project Management System (RPMS). Laboratory Facility staff are required to scan the cryovial and cryovial storage rack barcodes and upload the completed Supplemental Daily Activity and Saliva Sample Collection Form directly into the RPMS. A hard copy of this form should be stored in a locked cabinet for collection by the research team. This information will be used to ensure that the protocol is being followed appropriately and to assist sites with periodically shipping samples to The Florey Institute in Melbourne, Australia.

Refer to sample position layouts below as a guide (row A to be filled first, from position 1 to 12). Do not leave spaces blank.

Baseline Saliva samples:

	1	2	3	4	5	6	7	8	9	10	11	12
A	1A	1B	2A	2B	3A	3B	1A	2A	2B	3A	3B	3C
B	1A	1B	2A	2B	3A	3B	1A	2A	2B	3A	3B	3C
C	1A	1B	2A	2B	3A	3B	1A	2A	2B	3A	3B	3C
D	1A	1B	2A	2B	3A	3B	1A	2A	2B	3A	3B	3C
E	1A	1B	2A	2B	3A	3B	1A	2A	2B	3A	3B	3C
F	1A	1B	2A	2B	3A	3B	1A	2A	2B	3A	3B	3C
G	1A	1B	2A	2B	3A	3B	1A	2A	2B	3A	3B	3C
H	1A	1B	2A	2B	3A	3B	1A	2A	2B	3A	3B	3C

Month 2 Saliva samples:

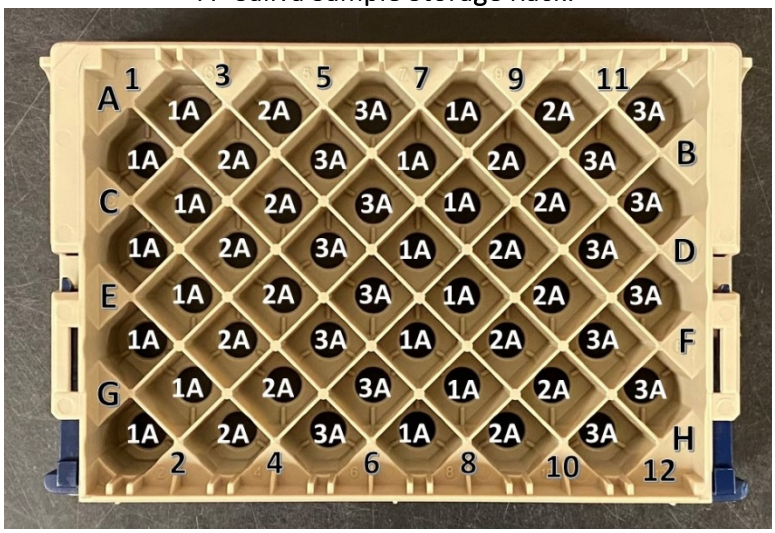
	1	2	3	4	5	6	7	8	9	10	11	12
A	1A	1B	2A	2B	3A	3B	1A	2A	2B	3A	3B	3C
B	1A	1B	2A	2B	3A	3B	1A	2A	2B	3A	3B	3C
C	1A	1B	2A	2B	3A	3B	1A	2A	2B	3A	3B	3C

ProNET

Sample storage racks are provided by the Genetics & Fluids core. The "A" samples (the first saliva sample collected at each of the three saliva sampling timepoints in a biomarker visit) will be stored separately from the "B" samples (the second saliva sample collected at each of the three saliva sampling timepoints). Each storage rack will be used to hold either the "A" or "B" saliva samples from **sixteen** biomarker visits. Samples will be stored as shown in the guide **below**. Note the following:

- Rows A, C, E, and G intersect with <u>even</u> numbers (e.g., A2, A4, A6, A8, A10, A12, etc.)
- Rows B, D, F, and H intersect with <u>odd</u> numbers (e.g., B1, B3, B5, B7, B9, B11, etc.)
- ***Please be extra attentive when recording the storage position of each sample to ensure that you are recording the correct storage position.***

"A" Saliva Sample Storage Rack:



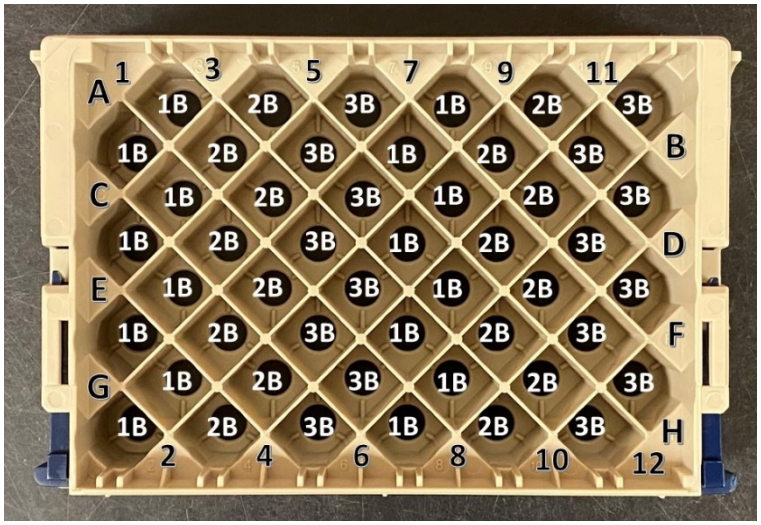
All of the "A" saliva samples (the first sample collected at each of the 3 saliva collection timepoints in a biomarker visit) will be stored in a single rack. Each saliva cryovial rack can hold the "A" saliva samples from 16 biomarker visits, with each row (A-H) holding the "A" saliva samples from 2 biomarker visits. Once a sample storage rack holds the "A" samples from 16 biomarker visits, a new "A" rack should be used.

If one or more saliva samples is not collected, please include the empty cryovial in the rack and record the volume as 0 mL in

D	1A	1B	2A	2B	3A	3B	1A	2A	2B	3A	3B	3C
E	1A	1B	2A	2B	3A	3B	1A	2A	2B	3A	3B	3C
F	1A	1B	2A	2B	3A	3B	1A	2A	2B	3A	3B	3C
G	1A	1B	2A	2B	3A	3B	1A	2A	2B	3A	3B	3C
H	1A	1B	2A	2B	3A	3B	1A	2A	2B	3A	3B	3C

the REDCap collection form. Make note of any SOP deviations in the comments section of the form.

"B" Saliva Sample Storage Rack:



All of the "B" saliva samples (the first sample collected at each of the 3 saliva collection timepoints in a biomarker visit) will be stored in a single rack. Each saliva cryovial rack can hold the "B" saliva samples from 16 biomarker visits, with each row (A-H) holding the "B" saliva samples from 2 biomarker visits. Once a sample storage rack holds the "B" samples from 16 biomarker visits, a new "B" rack should be used.

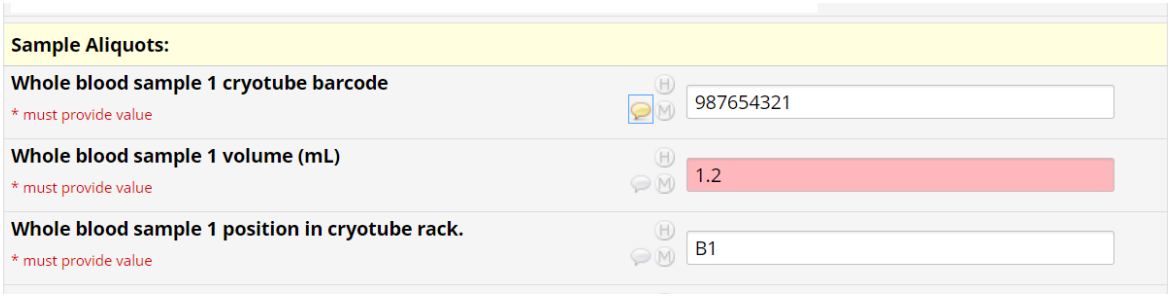
If one or more saliva samples is not collected, please include the empty cryovial in the rack and record the volume as 0 mL in the REDCap collection form. Make note of any SOP deviations in the comments section of the form.

The specific storage rack and position of each sample must be recorded on the Daily Activity and Saliva Sample Collection Form at time of collection and storage. This information will be used to ensure that the protocol is being followed appropriately and to assist sites with periodic sample shipments. Samples should not be moved or rearranged once they are stored in the sample storage racks.

SAMPLE STORAGE QC – ProNET Only

Both blood and saliva samples should periodically checked in the freezer to ensure correct storage procedures are being maintained. It's suggested that each time you place a newly processed cryovial into the freezer rack, you should check the other cryovials in the rack for any loose caps, cracks, or other deviations.

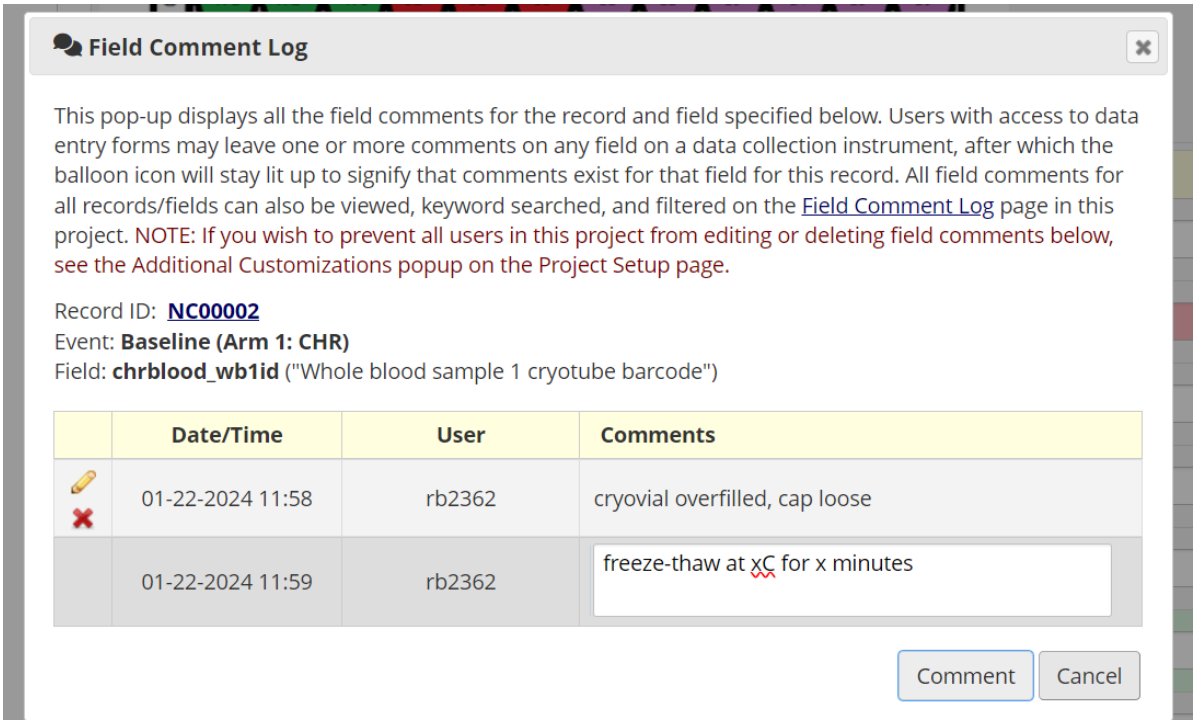
If any SOP deviations occur with the cryovials before, during, or after processing, please add a **field comment to the barcode** of the cryovial in REDCap and notify the Genetics and Fluids team ASAP. Click on the speech bubble button to the left of the barcode field, and enter the deviation into the comment.



Some examples include the following:

- "cryovial overfilled, cap loose"
- "cryovial cracked"
- "freeze-thaw at 22C for 60 minutes"

Multiple field comments can be added to one cryovial if necessary.



BLOOD AND SALIVA SAMPLE SHIPPING

PRESCIENT

Frozen blood and saliva samples will be shipped to The Florey Institute in Melbourne, Australia, approximately every 8 months to 1 year. Sites should make sure that they have enough freezer space to store samples for at least double this time. Sites will be responsible for costs associated with shipping.

Orygen will contact you when it is time for a shipment. They will work with you to prepare the shipment and to ensure that the samples are shipped securely.

ProNET

The blood sample storage racks containing frozen whole blood, serum, and plasma samples and the saliva sample storage racks containing frozen "B" saliva samples frozen blood and saliva samples will be batch shipped to the study biorepository approximately every 6-12 months. Sites must ensure that they have enough freezer space to store samples for at least double this time in case of delays.

The blood sample storage rack containing frozen buffy coat samples and the saliva sample storage rack containing frozen "A" saliva samples will be batch shipped at different times and to different places. Sites must ensure that they have enough freezer space to store these samples until they are asked to ship these samples.

Please contact the Genetics & Fluids core will contact you if you need to ship samples. The core will work with you to prepare the shipment and to ensure that the samples are shipped securely.

Inspecting the stored cryovials:

1. Prior to shipping inspect the cryovials.
2. Check to see that the caps are secure and there are no cracks in the cryovials.
    a. ProNET: if there are any issues with the cryovials, please refer to Sample Storage QC – ProNET only to properly handle and document them.
3. Ensure that all cryovials contain some sample. Empty cryovials should not be shipped.

Creating a shipment manifest:

1. Before they are shipped, a shipment manifest detailing the contents of the shipment is created. Please send a list of the barcodes for the boxes to UNC, and a manifest will be sent to you.
2. The manifest must be checked against the actual samples to be shipped for correctness prior to shipment. This should be performed with the samples kept on dry ice to ensure that they do not thaw.
3. Once you have confirmed that you have an accurate sample manifest, you will compile the shipment.

Shipping supplies:

1. 10 kg of dry ice in pellet form.
    a. If shipping more than 5 racks, please have another 3kg of dry ice on hand (total of 13kg).
2. Shipping materials:
    a. 13 x 13 x 12.5 insulated foam shipper
        i. Sonoco Thermosafe distributed by Fisher Scientific
        ii. Therapak from Avantor/VWR
            1. Therapak Comes with the IATA/DOT stickers necessary for shipping
        iii. It is important to note that this size shipper can only hold maximum 8 sample racks.
    b. Ziploc bags

    c. Absorbent material (paper towels, Kimwipes, cotton rounds)
    d. Tape
3. Shipping labels:
    a. For ProNET sites, please communicate your shipping timeline well in advance.
    b. They will need the following information to create a shipping label:
        i. Package dimensions
        ii. Approximate package weight (lb or kg)
        iii. Amount of dry ice used in kg
    c. A shipping label will be sent to sites via email. Sites will need to print and affix the label to the outside of the cardboard shipper.

Compiling the shipment (note that this will be completed by the shipping organization):

1. Place dry ice on the bottom of the insulated shipper.
2. Place each sample storage rack into a plastic Ziploc bag with some absorbent material such as paper towels. Securely seal the Ziploc bag, ensuring that excess air is not trapped in the bag when it is sealed.
3. Place the sealed bags containing sample storage racks in the middle of the shipper on top of the dry ice.
4. Place or pour dry ice on top of and on the sides of the sealed bags containing the sample storage racks. The sample storage racks should be covered on all sides.
5. Place the lid on the Styrofoam box. Ensure that there is no dry ice preventing the lid from fitting as it should. You may use 1 or two small pieces of tape to secure the lid to the shipper, but do not completely seal the shipper along the lid; gas must be able to escape as dry ice sublimates.
6. Place a printed copy of the completed sample manifest on top of the Styrofoam box within the cardboard box.
7. Use packaging tape to securely close the outer cardboard box. Ensure that a dry ice declaration sticker and either a sticker or writing indicating “Exempt Human Specimen” are displayed on the outer cardboard box. Follow any additional requirements of your institution or country.

Dry ice guidelines:

- Type of dry ice: Do not use large blocks of dry ice. Large blocks of dry ice may shift during shipment and break the sample storage racks or sample cryovials. It is best to use pellets of dry ice. If pellets of dry ice are not available, use a mallet or other tool to break the dry ice into small pieces before filling the insulated shipper.

- Amount of dry ice: Use enough dry ice to keep the shipment frozen for at least 48 hours, in case of shipment delay. The shipment should contain at least 10 kg of dry ice, or approximately 5 kg of dry ice for each 24 hour period.

Fluid Biospecimen FAQS

Please find the up-to-date version of the FAQs at this link: https://docs.google.com/document/d/1Xkyrek2nUkv-U71757njaAr8S6nJxS3CSrjbKyeUtsA/edit?usp=sharing

SUPPLY LIST

Procedure	Item	PRESCIENT	ProNET
Phlebotomy	No-additive tubes (3mL)	*To be purchased by site*	*Provided to sites from UNC; purchased from VWR and Thermo Fisher Scientific*
Phlebotomy	Red-top serum tubes (10 mL)	*To be purchased by site*	*Provided to sites from UNC; purchased from VWR and Thermo Fisher Scientific*
Phlebotomy	Purple-top K2EDTA tubes (3 mL)	*To be purchased by site*	*Provided to sites from UNC; purchased from VWR and Thermo Fisher Scientific*
Phlebotomy	Purple-top K2EDTA tubes (6mL)	*To be purchased by site*	*Provided to sites from UNC; purchased from VWR and Thermo Fisher Scientific*
Phlebotomy	Purple-top K2EDTA tubes (10 mL)	*To be purchased by site*	*Provided to sites from UNC; purchased from VWR and Thermo Fisher Scientific*
Blood Processing	1.4mL Cryovials and caps	*To be purchased by site*	*Provided to sites from UNC; purchased from Micronic*
Blood Processing	Colored caps for 1.4mL cryovials (green, red, purple, and blue)	*To be purchased by site*	*Provided to sites by UNC (with first shipment of kits only); purchased from Micronic*
Blood Processing/ Blood Storage	Transportation and storage racks for 1.4mL cryovials	*To be purchased by site*	*Provided to sites from UNC; purchased from Micronic*
Saliva Collection	Saliva Collection Aids[2] by Salimetrics	*To be purchased by site*	*Provided to sites from UNC; purchased from Salimetrics*
Saliva Collection	2.0mL Cryovials and caps	*To be purchased by site*	*Provided to sites from UNC; purchased from Thermo Fisher Scientific*
Saliva Collection/ Saliva Storage	Transportation and storage racks for 2.0mL cryovials	*To be purchased by site*	*Provided to sites from UNC; purchased from Thermo Fisher Scientific*
Batch Shipments	Insulated shipper	*Provided by courier or site*	*To be purchased by site*
Current Health Status Form	Physician scale with height rod	Suggested models include: ● Health O Meter 402KL ● Global Industrial Physician Beam Scale w/Height Rod	
Current Health Status Form	Blood pressure/heart rate monitor + child-size cuff	Suggested models include: ● Omron 5 Series Upper Arm Blood Pressure Monitor + Small D-Ring Cuff	
Current Health Status Form	No-Contact Infrared Thermometer	Suggested models include: ● ANKOVO AT-200 ● Visiomed LX-26E ● Berrcom JXB-178	
Phlebotomy/ Blood Processing/ Saliva Collection	Nitrile gloves	Use standard lab supplies; suggestion provided upon request	
Phlebotomy	21G Butterfly Needles	Use standard lab supplies; suggestion provided upon request	
Phlebotomy	Vacutainer Holders	Use standard lab supplies; suggestion provided upon request	
Phlebotomy	Tourniquets	Use standard lab supplies; suggestion provided upon request	
Phlebotomy/ Saliva Collection	Alcohol Pads	Use standard lab supplies; suggestion provided upon request	
Phlebotomy	Gauze Pads	Use standard lab supplies; suggestion provided upon request	
Phlebotomy	Medical Tape	Use standard lab supplies; suggestion provided upon request	
Phlebotomy	Sharps Containers	Use standard lab supplies; suggestion provided upon request	
Blood Processing	1000uL Pipette Tips	Use standard lab supplies; suggestion provided upon request	
Blood Processing	1D/2D/DataMatrix Barcode Scanner (with any needed accessories such as cradle or cable)	Suggested models include: ● Zebra DS8178-HC *recommended model ● Zebra DS4608-HC	

[2] Saliva Collection aids are a patented product created by Salimetrics. They are hard plastic tubing that click into 2mL cryovials and act as a straw for passive drool collection.

		● Zebra DS9908-HC
Blood Processing	Sterile 3mL disposable and sterile transfer pipettes	Use standard lab supplies; suggestion provided upon request
Blood Processing	15mL Sterile screw-top centrifuge tubes	Corning 352196
Blood Processing	Manual cryovial decapper	Suggested models include: ● Micronic tube decapper MP54000
Blood Processing	1000uL Multivolume pipettor	Use standard lab supplies
Blood Processing	Room-temperature centrifuge	Use standard lab supplies
Saliva Collection	Hard-Sided Sample Transportation Cooler	Suggested models include: ● Rubbermaid 530309

A. Biospecimen Collection Kits *(ProNET only)*

Some materials needed for this study will be provided in kits from the Genetics and Fluids Core at UNC. Core staff will assist with coordinating the very first shipment of kits to each site.

A single kit contains some of the blood and saliva materials needed for a single visit (baseline *OR* follow-up). Blood tubes will be grouped in bags by type, labelled with their individual expiration dates. Each site is responsible for adding the six blood collections tubes to one Biospecimen Collection Kit before each visit. Additional materials needed for each visit are listed in the Supply List and provided by the sites.

Each shipment of kits contains 16 kits, 5 bags of blood tubes, 2 storage boxes for blood sample cryovials, and 2 storage boxes for saliva sample cryovials. Two storage boxes for blood sample cryovials will hold exactly 16 visits' worth of whole blood, serum, and plasma samples, and 2 storage box for saliva storage cryovials will hold exactly 16 visits' worth of saliva samples.

Components of a Single Kit	
Item	**# of Units**
3mL no additive tube	1
10mL Serum tube	1
3mL EDTA tube	1
6mL EDTA tube	1
10mL EDTA tube	2
1.4mL barcoded cryotubes (for blood)	13
Saliva collection device	3
2mL barcoded cryotubes with caps (for saliva)	6

Components of a Shipment of Kits	
Item	**# of Units**
ProNET kits	16
3mL no additive tubes (16/bag)	1
10mL Serum tube (16/bag)	1
3mL EDTA tube (16/bag)	1
6mL EDTA tube (16/bag)	1
10mL EDTA tube (32/bag)	1
Storage rack for 1.4ml tubes (blood)	2
Storage rack for 2mL tubes (saliva)	2

Some additional materials will be included only in the very first shipment of kits a site receives only. Sites will be sent all of the 1.4mL cryovial caps they will need for the entire study, 1 additional rack to be used for frozen buffy coat sample storage, 1 additional rack to hold blood sample cryovials for visit preparation/transportation purposes, and 1 additional rack to hold saliva sample cryovials for visit preparation/transportation

- One (1) blood sample cryovial box will be used to store all of the collected and frozen buffy coat samples. If sites fill this storage box (96 biospecimen collection visits completed), contact UNC to receive another.
- One (1) blood sample cryovial box and one (1) saliva sample cryovial box are to be re-used at each visit as "Transportation racks." The purpose of these racks is to be used while preparing sample vials for study visits (e.g. while hand-labelling vials and scanning barcodes into the database) and for sample transportation.
- Caps should be kept in a sealed plastic bag and should always be handled with clean, gloved hands. Caps must not become contaminated.
- Sites must ensure that they do not accidentally discard any of these items.

Items included with the first shipment of kits ONLY ***Must be retained and used throughout the ENTIRE study***	
Item	**# of Units**
Green caps for 1.4mL cryotubes (blood)	>300
Red caps for 1.4mL cryotubes (blood)	>300
Purple caps for 1.4mL cryotubes (blood)	>600
Blue caps for 1.4mL cryotubes (blood)	>100
Storage rack for 1.4mL tubes (buffy coat)	1
Storage rack for 1.4ml tubes (blood transportation rack)	1
Storage rack for 2mL tubes (saliva transportation rack)	1

B. Production of Kits (*ProNET only)*

Prevent contamination of kit supplies:

- Always wear clean gloves when handling kit materials.
- Always reseal bags or containers of materials when not in use.

NOTE:	The following procedures describe the kit production procedures that take place at the ProNET Genetics & Fluids core only. These procedures do not apply to the ProNET sites.

How to make a single kit:

- The components of each kit must be checked for correctness by at least two people.
- Each kit (containing cryovials and saliva collection aids) must be contained within a sealed plastic bag.
- Each blood tube type must be contained within a sealed plastic bag and clearly labeled with the expiration date.
- If a single material is available in our stock with 2 different expiration dates, always use the materials expiring soonest (unless they're expiring *too soon!).*

How to make a shipment of kits:

- Each shipment of kits contains 16 kits, 5 bags of blood tubes, 2 blood sample storage racks, and 2 saliva sample storage racks.

- Label each of the two blood sample storage racks with freezer safe labels and then place them both into one plastic bag. Seal the plastic bag securely.
- Label each of the two saliva sample storage racks with freezer safe labels and then place them both into one plastic bag. Seal the plastic bag securely.
- Place the 16 bagged kits, 5 bagged blood tube types, bagged saliva storage racks, and bagged blood storage racks into a box. Add and place bubble wrap as needed to ensure that materials will not be damaged in transit.

Additional materials included in the very first shipment of kits only:

- In addition to the materials listed above, each site's very first shipment of kits will include one set of 1.4mL cryovial push caps (green, red, purple, and blue), one 1.4mL cryovial storage rack (for buffy coat samples), one 1.4mL cryovial transportation rack, and one 2mL cryovial transportation rack.
- Label the blood sample transportation rack with a freezer safe label.
- Label the blood sample (buffy coat) storage rack with a freezer safe label.
- Label the saliva sample transportation rack with a freezer safe label.
- Place all three racks into a plastic bag. Seal each plastic bag securely.
- Place the bagged set of 1.4mL cryovial push caps and the bagged set of racks (the 1.4mL cryovial transportation rack, the 2mL cryovial transportation rack, and the 1.4mL cryovial storage rack for buffy coat samples) into the box containing the 16 bagged kits, bagged saliva storage racks, and bagged blood storage racks. Add and place bubble wrap as needed to ensure that materials will not be damaged in transit.

C. Distribution of Kits (*ProNET only)*

Summary of Responsibilities

ProNET Coordination Team:

- Notifying the ProNET Genetics & Fluids core that a site is ready to receive their first shipment of kits (requires local IRB approval and sIRB submission)
- Obtaining the site's shipping address and communicating it to the ProNET Genetics & Fluids core

ProNET Genetics & Fluids Core:

- Producing the kits and shipments of kits
- Shipping the first shipment of kits to a site
- Providing the shipment tracking number and anticipated delivery date to the site
- Following up with the site to receive email confirmation of receipt and alerting the ProNET Coordination Team and the site PI to a lack of response if necessary
- Updating the ProNET Kit Shipment Tracker with the following information as it is available:
    - Date ProNET Coordination Team Confirmed Site Ready to Receive 1st Shipment of Kits
    - Shipping Address
    - Date Shipped
    - Tracking Number
    - Date Received
    - Recipient Name
    - Date of site request for kit shipment (Shipment 2 onward)

ProNET Sites:

- Tracking their on-site ProNET biospecimen kit inventory (including expiration dates)
- Notifying the ProNET Genetics & Fluids core when they are in need of another shipment of kits
- Providing updated an updated shipping address when requesting each shipment
- Assisting the ProNET Genetics & Fluids core with any international shipping requirements
- Tracking the shipment using the provided tracking number until receipt of shipment
- Confirming shipment contents and notifying the ProNET Genetics & Fluids core upon shipment receipt

The First Shipment of Kits

The ProNET coordination team and the ProNET Genetics & Fluids core are responsible for coordinating the first shipment of kits to each site. As the site nears recruitment readiness (e.g. obtains local IRB and Northwell sIRB approval), the ProNET coordination team will notify the ProNET Genetics & Fluids core that the site is ready to receive their first shipment of kits, and the ProNET Genetics & Fluids core will update the "ProNET Kit Shipment Tracker" to reflect this information.

Why can't all sites receive their kits at the same time? Blood collection tubes expire after a certain amount of time. The ProNET Genetics & Fluids core will only be shipping kits to a site as the site nears recruitment readiness and will keep in mind the study recruitment timeline when planning to ship kits to sites in order to minimize blood tube/kit wastage due to expiration.

Future Shipments of Kits

Sites will be responsible for ensuring that they always have a sufficient number of kits on hand and planning ahead when ordering more. After the first shipment of kits, the site is responsible for requesting additional shipments of kits. Sites should email the following individuals to request a shipment of kits. They will receive an acknowledgement of the request within 2 business days and the shipment of kits will be shipped to the site within 2 weeks of the request.

ProNET Kit Shipment Tracker

The "ProNET Kit Shipment Tracker" is located on a shared Google Drive. The document may be viewed by anyone with the link but can only be edited by the ProNET Genetics & Fluids core kit production team.

Shipping Addresses and Tracking Information

Sites will be asked to provide an updated shipping address each time they are shipped a shipment of kits, including the very first time. The shipment tracking number and the anticipated delivery date will be provided to the site once the shipment has been shipped. The tracking number and the date of shipment will be recorded in the "ProNET Kit Shipment Tracker." Sites should note that shipments may be delivered prior to or after the anticipated delivery date, so they should track the shipment continuously until shipment receipt. Once the site receives the shipment, they will be required to confirm receipt of the shipment and the presence of all kit components via email to the ProNET Genetics & Fluids core. The date the shipment is received, and the name of the shipment recipient will be recorded in the "ProNET Kit Shipment Tracker."

Site Location	Shipping Method	Shipping Time
North America	FedEx Express Saver	3 business days

Europe or Asia	FedEx International	5-7 business days depending on Import Customs

Sites outside of the US will be asked to assist the ProNET Genetics & Fluids core with compiling any needed customs documentation and with identifying the best method of shipping.

FAMILY INTERVIEW FOR GENETIC STUDIES (FIGS) FORM

- The FIGS is completed only at the screening visit.
- The FIGS is administered by study personnel who are certified to administer the Structured Interview for DSM 5 (SCID 5; please refer to the SOP concerning ascertainment / Teams B & I).
- The FIGS interview begins by determining the first-degree family members, including biological parents, full siblings, and children. Note that the demographic form also records whether the study participant has any children, so it may be useful to refer to this information while completing the FIGS.
- The interviewer then asks a series of questions designed to screen for depression, mania, and psychosis. Follow-up questions are only asked if the informant give a positive response to the screening questions for a family member.
- The FIGS is a semi-structured interview. The interview is very similar to the SCID, in that the SCID interviewer needs to ask each question, but the interviewer is free to word the question in a way that will best elicit the information and ask follow-up questions as needed to determine if the specific symptom of the disorder was likely to be present.
- Each question is answered with “yes,” “no,” or “unsure.” Scoring “yes” indicates the symptom was more likely than not to have been present. Scoring “no” indicates that the informant was likely to be in a position to have known that the symptom did not occur. Scoring “not sure” indicates that the informant wasn’t aware of the symptom but was not in a position to have necessarily known if the symptom actually occurred.
- Scoring rules are provided. The database automatically scores whether the disorder is “definitely”, “probably”, “possibly”, “not” present, or "unknown". However, scoring algorithms are imperfect, and the clinical rater may have additional information that could help inform the scoring of the FIGS. Thus, we ask the rater if s/he agrees or disagrees with scoring algorithm results. We recognize that there will be exceptions to the scoring algorithm, and it is important that we identify these exceptions.

HEALTH CONDITIONS FORM

- The Health Conditions Form is completed only at the screening visit.
- The Health Conditions Form is completed by the research assistant or the research clinician.
- The rater should have a basic familiarity with the medical conditions listed on the Health Conditions Form.
- The purpose is to determine whether the study participant has been given a previous medical diagnosis.
- Health conditions that are exclusionary for the study are indicated on the form.

General Start-Up Requirements

Each site must provide the **Genetics & Fluids Core** with their site-specific workflow, indicating which research staff member is responsible for each task and how information and data will flow between the research staff, phlebotomy staff, local hematology lab staff, and blood processing facility staff. Key issues include:

- Determination of the phlebotomy facility's requirements for tube labeling.
- Documentation of the site-specific protocol for obtaining CBC with differential: Determine the identity of the local lab that will perform routine hematology and procedures for sending the 3mL EDTA (purple top) tube to that lab. For example, the study could identify LabCorp as their local lab (LabCorp often discounts lab tests for research studies). In this case the Research Coordinator informs LabCorp when the blood specimen is obtained and places the blood specimen (following LabCorp procedures) in a LabCorp pick-up box for same-day pickup. The results of the test are sent electronically to the study team within a couple of days. Record whether the 3mL EDTA tube was sent to the local hematology lab. It is acceptable to work with any local lab that performs clinical hematology tests.
- Documentation of the site-specific protocol for transporting the blood specimens to the Blood Processing Facility.
- Documentation of how the laboratory staff processing the blood are to be informed regarding the blood processing protocol.
- Documentation of the procedures the site will use to obtain information needed for the Blood Sample Preanalytic Quality Assurance form. These items include:
    a. Date and time of blood collection
    b. Whether the 3mL EDTA tube was submitted for a CBC with Differential test
    c. Time specimen received in lab
    d. Centrifuge temperature
    e. Scanning cryotube barcodes into database
    f. Aliquoted volumes
    g. Presence of hemolysis
    h. Presence of lipemia
    i. Position in storage rack
    j. ID of storage rack
    k. ID of storage freezer
    l. Time samples place in freezer
    m. Problems encountered during sample processing or protocol deviations
- Documentation of the study-specific plan for saliva collection integration. Saliva is **collected at three time points separated by an hour. Saliva collection takes about 5 minutes**, thus other study procedures can be done in between saliva collection time points.
- Documentation of the procedures the site will use to monitor freezer temperatures.

A. Rater Training and Certification

- Staff will be given a copy of the detailed manual of standard operating procedures and instructed to familiarize themselves with the manual.
- We will do web-based training for all sections. The web-based training will include review of the written SOPs and pre-recorded videos of the protocols. The web-based trainings will be recorded and are to be viewed by new staff. Attendance to this training, or review of the recorded training, is to be completed before a study procedure is conducted for each staff member. Completion of this training will be documented.

- Research staff are to tape themselves performing the current health status protocol, including vital signs, with a confederate. Staff must be certified before performing these tasks with study participants.
- A "train the trainer" method will be implemented for sites using external phlebotomy labs and blood processing facilities (the lab manager/facility manager will need to be trained on our protocols and then will be responsible for ensuring that all lab staff who are tasked with performing our protocols are trained prior to doing so).
- Study Personnel will be certified on the FIGS and Health Conditions as part of their web-based trainings. The FIGS will be administered by a SCID-trained study staff member. The Health Conditions form will be completed by the same member of the study staff who completes the Medical/Psychiatrist History form at the screening visit.

Freezer Temperature Monitoring

- You will need a thermometer or other monitoring system for each of the freezers used for this study.
- If thermometers are to be used for temperature monitoring, they must be able to store at least 7 days of mix/max temperatures. The thermometers must be calibrated with calibration documentation available. Thermometers must not be used past the calibration expiration date. The Freezer Monitoring Temperature Log should be updated at minimum weekly with min/max freezer temperatures.
- If extended freezer monitoring systems are to be used for temperature monitoring, documentation (e.g. printouts) of extended temperature monitoring data may be maintained in lieu of Freezer Monitoring Temperature Logs.
- Upon discovery of a freezer temperature excursion, study staff must document the temperature excursion in the Freezer Temperature Excursion Log AND notify the Genetics & Fluids core of the temperature excursion.
    - ProNET sites must upload their Freezer Excursion Log Template to YaleBox before notifying the core.
- The freezer temperature may vary no more than by +/-15°C from -80°C (blood) or by more than +/-5°C from -20°C (saliva) and should remain at or near the set point, not near the minimum or maximum, the majority of the time.

	Minimum	Set Point	Maximum
Freezer	-25°C	-20°C	-15°C
Freezer	-35°C	-30°C	-20°C
Ultra-Low Freezer	-95°C	-80°C	-65°C

- Any excursions outside of these temperature ranges must be recorded on the Freezer Temperature Excursion Log. An extreme excursions that may affect sample quality will require more reporting.
    - **For ultra-low freezers, if the freezer reached above -20 °C for 1 hour or more**
      OR
    - **If a sample is about 0 °C for any length of time**
- If an extreme excursion occurs, record it on the Freezer Temperature Excursion Log as usual. Then, record it on the Protocol Deviation Log as a minor deviation and report it to study monitors. Finally, in REDCap you will need to add a field comment to *each* cryovial barcode indicating "freeze-thaw at **x**C for **x** minutes." For more info on field comments, please refer to Sample Storage QC – ProNET only.

ONGOING QC

A. ProNET/PRESCIENT Fluid Biospecimen Implementation Meeting

As of December 2022, Drs. Perkins and Phassouliotis will be hosting a monthly meeting to discuss implementation obstacles and frequently asked questions related to biospecimen collection and processing. They are held on the first Wednesday of the month at 6am EST and 3pm EST.

B. Fidelity to Study Protocols

Approximately three months after site initiation, research coordinators will be given a study procedure test that reviews key points and identifies protocol deviations. Research coordinators will be provided feedback based on the results of this test including re-training as needed. Research coordinators will be re-tested annually.

C. Data Monitoring

Value ranges are provided for most numerical data points. If entered values fall outside of the provided range, the data will be flagged for verification. In addition to the quality assurance checks implemented by the DPACC (e.g. ensuring that date of assessment after consent date), the following quality assurance checks will be implemented:

Form	Data Quality Checks
Crosscheck Reports	• Study Visit Dates of Current Health Status, Blood Preanalytics and Quality Assurance, and Complete Blood Count with Differential should match. • If Prenalaytic form indicates 3mL EDTA tube was sent to hematology lab for CBC w/diff, CBC w/diff results must be available.
FIGS	• Age of siblings and children must be less than age of parents.
Current Health Status Flags	• BMI must be greater than or equal to 14 and less than or equal to 59. • Systolic BP must be greater than diastolic BP. • Activity dates (sleep, wake, eat, used tobacco, used marijuana, last menstrual period) must occur prior to assessment date. • Date of last symptoms, date of last COVID-19 symptoms, date of COVID-19 vaccine dose 1 and dose 2 (if applicable) must occur prior to assessment date.
Blood Sample Preanalytic Quality Assurance Flags	• Time to lab must be after time of blood draw. • Time to freezer must be after time to lab and time of blood draw. • Sample barcodes must not be duplicates (checking within the form to ensure that the same cryovial barcode isn't scanned twice in error). • Cryovial volume must not be above 1mL.
Blood Sample Preanalytic Quality Assurance Reports	• Report on visits where centrifuge temperatures were ~5°C more or less than 20°C are flagged. • Report on visits where processing time is greater than 90 minutes or less than 30 minutes.
CBC with Differential Flags	• Values outside of reference range matched to sex assigned at birth. • Sum of Baso, Eos, Mono, Lymph, Neut must equal WBC (+/- 0.1).
Daily Activity and Saliva Collection Flags	• Activity dates (sleep, wake) must occur prior to assessment date. • Date saliva samples were collected must not occur after assessment date. • Saliva timepoints 1, 2, and 3 must occur in chronological order. • Time saliva samples were frozen must occur after saliva timepoints 1, 2, and 3.

APPENDIX

A. Family Interview for Genetic Studies (FIGS) V1.7

Interviewer Instructions

The FIGS interview begins by determining the first-degree family members, including biological parents, full siblings, and children. Note that the demographic form also records whether the subject has any children, so it may be useful to refer to this information while completing the FIGS.

The interviewer then asks a series of questions designed to screen for depression, mania, and psychosis in each first-degree family member. Follow-up questions are only asked if the informant gives a positive response to the screening questions for a family member.

The FIGS is a semi-structured interview. Each symptom should be addressed but the interviewer is free to word the question in a way that will best elicit the information and ask follow-up questions as needed to determine if the specific symptom of the disorder was likely to be present.

Each question probes for one or more DSM 5 symptom criteria. are scored as “yes,” “no,” or “unknown”. Scoring “yes” indicates the symptom was more likely than not to have been present at a severity sufficient to meet the corresponding DSM 5 criteria. Scoring “no” indicates that the informant was likely to have known that the symptom did not occur. Scoring “unknown” indicates that the informant was not in a position to have known if the symptom actually occurred.

Second-hand information is allowable. It is not required that the subject have witnessed the behavior or heard the family member report the symptom. For example, the subject may relate that they had never met their father but had heard from their mother that their father had a diagnosis of schizophrenia (P18 scored "yes"), would talk to people that weren't really there (P8 scored "yes"), and had left town because he thought the mayor and police chief had a vendetta against him (P1 scored "yes") but not know anything more about their father's illness. In this scenario the remaining psychosis would then be scored "unknown".

Diagnostic scoring rules for "Depression", "Mania", "Psychosis", "Non-Affective Psychosis" and "Affective Psychosis" are provided at the end of this document. The database automatically determines whether the disorder is "definitely", "probably", "possibly", or not present ("absent") or if there is "insufficient information" to assess based on these scoring rules. However, scoring algorithms may be imperfect, and the clinical rater may have additional information that could help inform the scoring of the FIGS. Thus, we ask the rater if they agrees or disagrees with scoring algorithm results. We recognize that there may be exceptions to the scoring algorithm, and it is important that we identify these exceptions.

Interview date:	_____/___/___ YYYY/DD/MM

We will collect information on your close biological family members, including your mother, father, siblings (from the same mother and father), and children. We would like to better understand your family members' mental health history, including history of mental illness diagnosis, treatment, or symptoms of mental illness. To begin…

Do you know this kind of information about your biological mother? (For example, did you grow up with/live with/spend time with or hear about her?)

Do you know this kind of information about your biological father? (For example, did you grow up with/live with/spend time with or hear about him?)

How many biological siblings (people who have the same biological mother and biological father as you do) do you have?

Do you know this kind of information about (sibling) (for example did you grow up with/live with/spend time with or hear about him/her)?

How many biological children do you have?

Do you know this kind of information about (child) (for example did you grow up with/live with/spend time with or hear about him/her)?

Record known first degree biological relatives in shaded cells. (Mother=M, Father=F, Sibling 1-Sibling 9 = S1-S9, Child 1=Child 4 = C1-C41)	M	F				
Record sex of biological relative (male=1, female=2)	2	1				
Record age of biological relative (if deceased, age at death).						
Record if participant knows information about biological relative (no=0, yes=1)						

Record biological family member about whom the participant indicates they have knowledge in shaded cells at beginning of each section.

(Not Scored) Begin by making a general inquiry about whether any of these family members ever treated for problems with their nerves or emotions: Were any of these family members ever hospitalized or did they ever receive psychotherapy, a medication, or any other intervention (for mental health issues)?
(Next, let the participant know that you are going to continue with more specific (scored) questions.)

Item	**Question**	Record biological family member about whom the participant indicates they have knowledge in shaded cells						**Skip-Out Instructions**
S01	Did any of these family members talk of death or suicide?	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	*If yes, ask depression follow-up questions.*
S02	Did any of these family members attempt or complete suicide?	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	*If yes, ask depression follow-up questions.*
S03	Did any of these family members ever have any of the following: feel very low for a couple of weeks or more, have a diagnosis of depression, or receive any treatment (like a medication, psychotherapy, hospitalization, etc.)?	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	*If yes, ask depression follow-up questions.*
S04	Did any of these family members ever seem overexcited (or manic), have a diagnosis of mania or bipolar disorder, or receive any treatment (like a medication, psychotherapy, hospitalization, etc.)?	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	*If yes, ask mania follow-up questions.*
S05	Did any of these family members ever have visions, hear voices, or have beliefs that seem strange or unreal?	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	*If yes, ask psychosis follow-up questions.*
S06	Did any of these family members ever have unusual or bizarre behavior, have a diagnosis of schizophrenia or	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	*If yes, ask psychosis*

	schizoaffective disorder, or receive treatment (such as a medication, psychotherapy, hospitalization, etc.)?							*follow-up questions.*
S07	Did any of these family members ever speak in a way that was hard to follow or didn't make any sense?	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	*If yes, ask psychosis follow-up questions.*
S08	Were any of these family members ever extremely jealous, or suspicious, or believe in magic, or see special meanings in things that no one else saw?	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	*If yes, ask psychosis follow-up questions.*

Depression Follow-Up Questions								
		Record biological family member who had an answer of "yes" to screening questions S01, S02, or S03						
D01	During your family member's depression was he/she depressed most of the day, nearly every day, for as long as two weeks or more?	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	*If both D01 and D02 are scored "no" or "unknown," skip to D11.*
D02	During your family member's depression did he/she lose interest in things or become unable to enjoy most things, for as long as two weeks or more?	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	
D03	During your family member's depression did he/she have a change in appetite or lose weight without trying to?	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	
D04	During your family member's depression did he/she have a change in sleep patterns (either too much or too little)?	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	
D05	During your family member's depression did he/she move or speak more slowly than usual? During your family member's depression did he/she pace or wring his/her hands?	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	
D06	During your family member's depression did he/she have less energy or feel tired out?	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	
D07	During your family member's depression did he/she feel guilty, worthless or blame himself/herself?	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	
D08	During your family member's depression did he/she have trouble concentrating or making decisions?	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	
D09	During your family member's depression did he/she talk about suicide or attempt suicide?	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	
D10	During your family member's depression, did he/she have difficulty with/become	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	

	unable to work, go to school, or take care of household responsibilities? Score "yes" if meets severity criteria for major depression, e.g., cause the individual clinically significant distress or impairment in social, occupational, or other important areas of functioning).							
D11	Was your family member ever given a diagnosis of major depression or receive treatment for major depression (such as receiving an antidepressant medication, psychotherapy, or hospitalization, etc.)? *Do not score therapy for other reasons, such as support for coping with stressful life events or counseling for other reasons such as marital therapy.*	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	
D12	During your family member's depression did he/she have visions, or hear voices, or have beliefs or behavior that seem strange or unusual, at the same time as his/her other symptoms of depression?	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	*If yes, ask psychosis follow-up questions*

Mania Follow-Up Questions								
		Record biological family member who had an answer of "yes" to screening questions S04						
M01	For most of the time over several days (no need to include time frame if hospitalized), did your biological family member ever seem hyper or wired or much more active/have an unusual amount of energy (more than usual) or have abnormally or persistently elevated goal-directed behavior (more than usual)?	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	*If M01, M02, and M03 scored "unknown," or "no" skip to M11*
M02	For most of the time over several days did your biological family member ever seem too happy/high/excited (more than usual)?	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	
M03	For most of the time over several days did your biological family member ever act very irritable or angry (more than usual)?	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	
M04	For most of the time over several days did your biological family member ever feel that he/she had special gifts or powers or inflated self-esteem (more than usual)?	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	
M05	For most of the time over several days did your biological family member ever	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	

	need less sleep without feeling tired (more than usual)?							
M06	For most of the time over several days did your biological family member ever become more talkative than usual or pressure to keep talking (more than usual)?	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	
M07	For most of the time over several days did your biological family member ever jump from one idea to another (more than usual) or said his/her thoughts were racing?	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	
M08	For most of the time over several days did your biological family member ever become easily distracted (more than usual)?	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	
M09	For most of the time over several days did your biological family member ever get involved in too many activities at work, at school, or socially (more than usual)?	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	
M10	For most of the time over several days did your biological family member ever show poor judgment (e.g., spending sprees, sexual indiscretions) or behave in such a way as to cause difficulty for those around his/her (obnoxious/manipulative) (more than usual)?	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	
M11	Did your biological family member ever get a diagnosis of mania, bipolar disorder, or manic-depressive disorder, or receive treatment for the symptoms [such as a mood stabilizer medication (such as lithium) or hospitalization]?	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	
M12	For most of the time over several days did your biological family member ever have visions, or hear voices, or have beliefs or behavior that seem strange or unusual, at the same time as his/her other symptoms of mania?	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	*If M12 yes, ask psychosis follow-up questions*

Psychosis Follow-Up Questions							
		Record biological family member who had an answer of "yes" to screening questions S05, S06, or S07, S08, D12, M12					
P01	Did your family member ever believe people were following or that someone was trying to hurt or poison him/her? (Determine if symptom met psychosis severity criteria and if symptom was present for a significant proportion of time for at least a one-	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**

	month period. Only score "yes" if symptom met psychosis severity criteria.)						
P02	Did your family member ever believe someone was reading his/her mind? (Determine if symptom met psychosis severity criteria and if symptom was present for a significant proportion of time for at least a one-month period. Only score "yes" if symptom met psychosis severity criteria.)	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**
P03	Did your family member ever believe he/she was under the control of some outside person or power or force? (Determine if symptom met psychosis severity criteria and if symptom was present for a significant proportion of time for at least a one-month period. Only score "yes" if symptom met psychosis severity criteria.)	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**
P04	Did your family member ever believe his/her thoughts were broadcast, or that an outside force took away his/her thoughts or put thoughts into his/her head? (Determine if symptom met psychosis severity criteria and if symptom was present for a significant proportion of time for at least a one-month period. Only score "yes" if symptom met psychosis severity criteria.)	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**
P05	Did your family member ever find special meaning in TV, radio, or newspaper articles (or internet/social media)? (Determine if symptom met psychosis severity criteria and if symptom was present for a significant proportion of time for at least a one-month period. Only score "yes" if symptom met psychosis severity criteria.)	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**
P06	Did your family member ever have any other strange or unusual beliefs? (May score delusional ideation revealed during screening here if not already scored in P01-P05). (Determine if symptom met psychosis severity criteria and if symptom was present for a significant proportion of time for at least a one-month period. Only score "yes" if symptom met psychosis severity criteria.)	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**
P07	Did your family member ever see things that were not really there? (Determine if symptom met psychosis severity criteria and if symptom was present for a significant proportion of time for at least a one-month period. Only score "yes" if symptom met psychosis severity criteria.)	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**

P08	Did your family member ever hear voices or other sounds that were not real? (Determine if symptom met psychosis severity criteria and if symptom was present for a significant proportion of time for at least a one-month period. Only score "yes" if symptom met psychosis severity criteria.)	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**
P09	Did your family member ever speak in a way that was difficult to follow or didn't make much sense? (Determine if symptom met psychosis severity criteria and if symptom was present for a significant proportion of time for at least a one-month period. Only score "yes" if symptom met psychosis severity criteria.)	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**
P10	Did your family member ever seem to be physically stuck in one position, or move around excitedly without any purpose? (Determine if behavior was grossly disorganized or catatonia. Only score "yes" if symptom met psychosis severity criteria.)	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**
P11	Did your family member ever do something peculiar like talking to his or herself in public? (Determine if symptom met psychosis severity criteria and if symptom was present for a significant proportion of time for at least a one-month period. Only score "yes" if symptom met psychosis severity criteria.)	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**
P12	Do you generally know about your family member's different medical conditions, or if they may have used drugs? (If no, score as "Unknown") Has your family member used drugs? (If yes to drugs) Did the issues/behaviors we just talked about occur when they were NOT high or on drugs? Does your family member have a health condition that might have caused these issues/behaviors (for example dementia, Parkinson's disease, Alzheimer's disease, Huntington's disease, autoimmune thyroid disease)? (If yes to neurological/medical problem) Did the issues/behaviors we just talked about start (at least several years) BEFORE the (neurological disease) started? (Determine if the psychosis symptoms cannot be better explained by an organic cause. Score "Yes" if: -the family member did not use drugs and did not have a serious medical/neurological problem. -the psychosis symptoms occurred when the person was not high on drugs	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**

	-the family member did not have a possible contributing medical/neurological condition -the symptoms developed prior to any medical/neurological disorder that may involve psychosis as a symptom. Score "No" if symptoms only occurred when the person was high on drugs and/or if symptoms developed as part of a medical/neurological disorder.)						
P13	Did your family member ever appear to have noticeably reduced emotions, or inappropriate emotions? (Determine if symptom met negative symptom severity criteria and if symptom was present for a significant proportion of time for at least a one-month period. Only score "yes" if symptom met negative symptom severity criteria.)	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**
P14	Did your family member ever have few interests, reduced drive or energy? Determine if symptom met negative symptom severity criteria and if symptom was present for a significant proportion of time for at least a one-month period. Only score "yes" if symptom met negative symptom severity criteria.)	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**
P15	Did your family member ever neglect hygiene and grooming? Determine if symptom met negative symptom severity criteria and if symptom was present for a significant proportion of time for at least a one-month period. Only score "yes" if symptom met negative symptom severity criteria.)	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**
P16	Did your family member ever often be at a loss for words or not join in conversations (consistent with poverty of content, e.g., alogia)? Determine if symptom met negative symptom severity criteria and if symptom was present for a significant proportion of time for at least a one-month period. Only score "yes" if symptom met negative symptom severity criteria.)	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**
P17	How much of the time was your family member depressed/manic? Was your family member depressed/manic for the minority of the time that they were having the (psychotic disorder symptoms/behaviors)? (Determine if depression/mania episodes were present for a minority of the total duration of the	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**

	psychotic disorder. Score 'Yes' if depression/mania symptoms present for a minority, and 'no' if depression/mania symptoms present for most of the illness.)						
P18	Did your biological family member ever get a diagnosis of schizophrenia, schizophreniform or schizoaffective disorder, or receive treatment for these symptoms (such as an antipsychotic medication, or hospitalization), (unrelated to drugs/medical condition)? [P18 is only scored "yes" if the diagnosis/treatment involved symptoms/behaviors not caused by drugs or medical conditions. If P12 -Organic Cause item is scored "No" (psychosis symptoms explained by drugs/medical condition), determine whether the diagnosis/treatment involved episodes outside of those already discussed. If the diagnosis/treatment only included episodes that were likely caused by drugs/medical condition, then score this item, P18 "No". If the diagnosis/treatment involved episodes that were unlikely to be caused by drugs/medical condition, and/or the study participant lacks knowledge about the circumstances related to the diagnosis/treatment, then score this item, P18, "Yes".]	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**	**Y N UK**

Psychosis Scoring Rules						
Insufficient Information	At least one of S5, S6, S7, ***and*** S8 scored "unknown", and the others scored "no" or "unknown", ***or*** P12 scored "unknown" ***and*** P18 scored "unknown"					
Definite	One or more of P1-P11 scored “yes” ***and*** P12 scored “yes”					
Probable	One or more of P1-P11 scored “yes” ***and*** P12 scored “unknown” ***and*** P18 scored “yes”					
Possible	Only P18 scored “yes”					
Absent	Does not meet criteria for “Insufficient Information” ***or*** “Definite” ***or*** “Probable” ***or*** “Possible” Psychosis					
Family Member:						
Psychosis **(needed for PSYCHS)**	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**

Family member:						
Major Depression	**3 = Definite 2 = Probable 1 = Possible**	**3 = Definite 2 = Probable 1 = Possible**	**3 = Definite 2 = Probable 1 = Possible**	**3 = Definite 2 = Probable 1 = Possible**	**3 = Definite 2 = Probable 1 = Possible**	**3 = Definite 2 = Probable 1 = Possible**

	0 = Absent 9 = Insufficient information	**0 = Absent 9 = Insufficient information**	**0 = Absent 9 = Insufficient information**	**0 = Absent 9 = Insufficient information**	**0 = Absent 9 = Insufficient information**	**0 = Absent 9 = Insufficient information**
Mania	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**
Psychosis	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**
Schizophrenia	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**
Affective Psychotic Disorder	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**	**3 = Definite 2 = Probable 1 = Possible 0 = Absent 9 = Insufficient information**

Scoring Rules: Major Depression, Mania, Psychosis, Schizophrenia, Affective Psychotic Disorders	
Major Depression	
Insufficient Information	Study participant unable to provide information regarding depression to score (#S1 ***and*** #S2 scored "no" or "unknown" ***and*** #S3 scored "unknown" ***or*** #D1, #D2, ***and*** #D10 scored "unknown")
Definite	Either #D1 ***or*** #D2 ***and*** four or more of items: #D1-#D8 ***and*** item #D9 scored "yes"
Probable	Either #D1 ***or*** #D2 ***and*** two or more of items #D1-#D8 ***and*** item #D9 scored "yes"
Possible	Either #D1 ***or*** #D2 ***and*** two or more of items #D1-#D8 ***and*** item #D9 scored "unknown"
	Only item #D10 scored "yes"
Absent	Does not met criteria for definite, probable, or possible depression
Mania	
Insufficient Information	Study participant unable to provide information regarding mania to score (#S4 scored "unknown" ***or*** #M1 ***and*** #M11 "unknown" ***or*** #M2 ***and*** #M3 ***and*** #M11 "unknown***"***)
Definite	Items #M1 ***and*** item #M2 ***or*** #M3 ***and*** four or more of items #M4-#M10 scored "yes"
Probable	Items #M1 ***and*** item #M2 ***or*** #M3 ***and*** two or more of items #M4-#M10 scored "yes"
Possible	Only item #M11 scored "yes"
Absent	Does not met criteria for definite, probable, or possible mania

Psychosis	
Criteria for the five symptom domains:	
Delusions:	*One or more of Items #P1-#P6 scored "yes"*
Hallucinations:	*One or more of Items #P7-#P8 scored "yes"*
Disorganization:	*Item #P9 scored "yes"*
Disorganized behavior:	*One or more of Items #P10-#P11 scored "yes"*
Negative symptoms:	*One or more of Items #P13-#P16 scored "yes"*
Insufficient Information	Study participant unable to provide information regarding psychosis to score (#S5, #S6, #S7, ***and*** #S8 scored "unknown")
Definite	One or more of items #P1- #P11 scored "yes" ***and*** item #P12 scored "yes"
Probable	One or more of items #P1-#P11 scored "yes" ***and*** item #P12 scored "unknown"
Possible	Only item #P18 scored "yes"
Absent	Does not meet criteria for definite, probable, or possible psychosis
Non-Affective Psychotic Disorder (Schizophrenia)	
Insufficient Information	Study participant unable to provide information regarding non-affective psychotic disorder to score (#S5, #S6, #S7, ***and*** #S8 "unknown" ***or*** they DO meet criteria for definite, probable, or possible psychosis ***and*** only 1 symptom domain is met ***and*** AT LEAST 1 other symptom domain is unknown)
Definite	Definite psychosis ***and*** meets criteria for at least two of the five symptom domains ***and*** item #P17 <u>not</u> scored "no"
Probable	Definite or probable psychosis ***and*** item #P17 scored **either** "not applicable" **or** "unknown"
Possible	Only item #P18 only scored "yes"
Absent	Does not meet criteria for definite, probable, or possible psychosis
Affective Psychotic Disorder	
Insufficient Information	Study participant unable to provide information regarding non-affective psychotic disorder to score ("insufficient information" for both depression diagnosis and mania diagnosis)
Definite	Item #P17 scored "no" **and** Definite Depression **and** Item #D11 "yes" **or** Definite Mania **and** item #M12 scored "yes"
Probable	Item #P17 scored "no" **and** Probable Depression **and** Item #D11 "yes" **and** no Definite Mania **with** item #M12 "yes" **or** Probable Mania **and** item #M12 "yes" **and** no Definite Depression **with** item #D11 "yes"
Possible	Item #P17 scored "no" **and** Possible Depression **and** Item #D11 "yes" **and** no Definite **or** Probable Mania **with** item #M12 "yes" **or** Possible Mania **and** item #M12 "yes" **and** no Definite **or** Probable Depression **with** item #D11 "yes"
Absent	Does not meet criteria for definite, probable, or possible psychosis

B. Health Conditions Form

Health Conditions Form			
Interview Date			
Ask the study participant each of the general questions in **bold.** If the study participant answers "yes," determine the specific condition. *NOTE: A serious medical condition may be exclusionary. Potentially exclusionary neurological conditions are denoted by asterisks (*), but any serious medical problem could be exclusionary – when in doubt, discuss with the study team.*			
"Do you have any problems with your immune system, such as allergies or an autoimmune disorder?" **SLE (lupus) scored under skin rashes* **Autoimmune thyroid problem (e.g. Hashimoto's) scored under endocrine problems*	**Yes**	No	**Unknown**
Food allergies	Yes	No	N/A
Hives	Yes	No	N/A
Seasonal allergies	Yes	No	N/A
Other allergies (describe):	Yes	No	N/A
"Any problems with your breathing? How about problems with asthma?"	**Yes**	No	**Unknown**
Asthma	Yes	No	N/A
Other lung problem (describe):	Yes	No	N/A
"How about arthritis?"	**Yes**	No	**Unknown**
Osteoarthritis	Yes	No	N/A
Rheumatoid arthritis	Yes	No	N/A
Other arthritis (describe):	Yes	No	N/A
"How about problems with diabetes, your thyroid, or other hormone problems?"	**Yes**	No	**Unknown**
Diabetes type 1	Yes	No	N/A
Diabetes type 2	Yes	No	N/A
Autoimmune thyroid problem (e.g. Hashimoto's)	Yes	No	N/A
Non-autoimmune thyroid problem	Yes	No	N/A
Other endocrine problem (describe):	Yes	No	N/A
"How about a blood condition like hemophilia or von Willebrand's? How about high cholesterol or triglycerides?"	**Yes**	No	**Unknown**
Hemophilia	Yes	No	N/A
Von Willebrand disease	Yes	No	N/A
High cholesterol	Yes	No	N/A
High triglycerides	Yes	No	N/A
Other blood condition (describe):	Yes	No	N/A
"How about any developmental disorders, like ADHD, learning disabilities, or autism?"	**Yes**	No	**Unknown**
Attention deficit hyperactivity disorder (ADHD)	Yes	No	N/A
Autism Spectrum Disorder	Yes	No	N/A
Learning disabilities	Yes	No	N/A
Other developmental problem (describe):	Yes	No	N/A
"How about problems with rashes like eczema or psoriasis? Any other rashes?"	**Yes**	No	**Unknown**

Eczema	Yes	No	N/A
Psoriasis	Yes	No	N/A
SLE (lupus)	Yes	No	N/A
Other skin rash (describe):	Yes	No	N/A
"How about any heart conditions?"	**Yes**	No	**Unknown**
Cardiovascular disease/heart disease	Yes	No	N/A
Coronary artery disease	Yes	No	N/A
Myocarditis	Yes	No	N/A
Other heart condition (describe):	Yes	No	N/A
"How about GI problems like Celiac, Crohn's disease, irritable bowel syndrome, or gastritis?"	**Yes**	No	**Unknown**
Celiac disease	Yes	No	N/A
Crohn's disease	Yes	No	N/A
Gastritis	Yes	No	N/A
Irritable bowel syndrome (IBS)	Yes	No	N/A
Ulcerative colitis	Yes	No	N/A
Other GI problem (describe):	Yes	No	N/A
"How about problems with migraines, tension headaches, seizures, or other neurological problems?"	**Yes**	No	**Unknown**
Migraine headaches	Yes	No	N/A
Multiple sclerosis*	Yes	No	N/A
Seizures*	Yes	No	N/A
Tension headaches	Yes	No	N/A
Concussion/traumatic brain injury*	Yes	No	N/A
Other neurological problem (describe)*:	Yes	No	N/A
"How about a chronic viral infection like hepatitis, herpes, HIV, or mono?"	**Yes**	No	**Unknown**
Hepatitis A	Yes	No	N/A
Hepatitis B	Yes	No	N/A
Hepatitis C	Yes	No	N/A
HIV/AIDS*	Yes	No	N/A
HSV-1 ("oral herpes" or "cold sores")	Yes	No	N/A
HSV-2 ("genital herpes")	Yes	No	N/A
Mononucleosis	Yes	No	N/A
Other viral infection (describe):	Yes	No	N/A
"How about cancer?"	Yes	No	N/A
"How about sleep apnea?"	Yes	No	N/A
"Any signs of gingivitis or periodontitis?"	Yes	No	N/A

"How about polycystic ovary syndrome (PCOS)?"	Yes	No	N/A

C. Health Conditions Reference Form

Health Conditions Reference Sheet	
Health Condition	**Description**
Food allergies	A food allergy is an immune system reaction that occurs soon after eating a certain food (e.g. peanuts). Symptoms include hives, swollen airways, and trouble breathing. Severe symptoms, or anaphylaxis, are life-threatening. Food intolerance (difficulty digesting certain foods such as **lactose intolerance**) is coded under "other GI problems" rather than here.
Hives	Hives, (**urticaria**) is a skin reaction that are itchy welts, which range in size from small spots to large blotches. Hives can be triggered by many situations and substances, including exposure to certain foods or medications.
Seasonal allergies	Seasonal allergies (**hay fever, allergic** rhinitis) are allergic reactions to pollen, mold, etc. that result in runny nose, watery eyes, etc.
Other allergies (describe):	Allergies are a specific type immune system reaction to a foreign substance that affect airways, skin, sinuses, or the digestive system.
Asthma	Asthma is when airways narrow and swell and (sometimes) produce extra mucus. Breathing, especially exhaling, is difficult, triggering coughing, wheezing, and shortness of breath.
Other lung disorders	Acute respiratory disorders (e.g. infectious such as influenza) are not coded here.
Osteoarthritis	Osteoarthritis involves joint pain, swelling, and disfigurement resulting from loss of cartilage (the cushion between bones). Adolescents and young adult may develop osteoarthritis after an injury to a joint.
Rheumatoid arthritis	Rheumatoid arthritis is an autoimmune disorder involving joint pain, swelling, and disfigurement. Rheumatoid arthritis often affects other parts of the body, including the skin, eyes, lungs, heart and blood vessels. Symptoms may start in childhood (**Juvenile arthritis)** with symptoms that also affect growth.
Other arthritis (describe):	Arthritis that are part of other disorders (**SLE, psoriasis**) are also coded here. Rare forms of arthritis include **ankylosing spondylitis (hereditary**). Arthritis may also be a complication of infectious diseases such as **Lyme disease** and **Brucellosis (cat scratch fever),** to name a few.
Diabetes type 1	Type 1 diabetes, once known as **juvenile diabetes or insulin-dependent diabetes**, is a chronic condition in which the pancreas produces little or no insulin. Insulin is a hormone needed to allow sugar (glucose) to enter cells to produce energy.
Diabetes type 2	Type 2 diabetes is an impairment in the way the body regulates and uses sugar (glucose) as a fuel. This long-term condition results in too much sugar circulating in the bloodstream. Eventually, high blood sugar levels can lead to disorders of the circulatory, nervous, and immune systems. Type 2 diabetes used to be known as adult-onset diabetes, but both type 1 and type 2 diabetes can begin during childhood with obesity a risk factor.
Autoimmune thyroid problem (e.g. Hashimoto's)	Hashimoto's disease is an autoimmune disease, where the immune system attacks the thyroid gland, causing low levels of thyroid hormone. **Symptoms of untreated thyroid disease include psychosis as well depression, irritability, anxiety, fatigue**.
Non-autoimmune thyroid problem	Numerous, rare disorders may affect the thyroid gland in children an young adults, often causing too much thyroid to be released. Disorders include **thyroiditis** (**inflammation in the thyroid), Graves'** disease (an autoimmune disorder), and **thyroid nodules.**
Other endocrine problem (describe):	The most common feature of other endocrine disorders in children involve delayed development/delayed puberty. Examples include genetic disorders (**Turner syndrome in girls, Klinefelter syndrome in boys, Kallmann syndrome**) and also **Addison's disease (adrenal insufficiency**). Low levels of **Vitamin D** may cause **rickets** involving the softening and weakening of bones in children.
Hemophilia	Hemophilia is a severe blood clotting disorder caused by lack a coagulation factor. People with hemophilia bleed easily.
Von Willebrand disease	Von Willebrand disease is a less severe clotting disorder. Symptoms often include heavy menses, frequent nose bleeds, and easy bruising.

High cholesterol	Cholesterol levels are measured with a specific blood test. Cholesterol is required by the body, but elevated levels are a risk factor for cardiovascular disease.
High triglycerides	Triglyceride levels are measured with a specific blood test. Triglycerides are required by the body, but elevated levels are a risk factor for cardiovascular disease.
Other blood condition (describe):	There is a long list of blood disorders. Examples include blood disorders due to genetic factors (**thalassemia, sickle cell anemia**) and autoimmune blood disorders [**immune thrombocytopenia (ITP)** and **autoimmune hemolytic anemia**.)
Attention deficit hyperactivity disorder (ADHD)	ADHD includes inattention and hyperactive-impulsive behavior, emerging before age 12.
Autism Spectrum Disorder	Autism spectrum disorder affects how a person perceives and socializes with others, causing problems in social interaction and communication. The disorder also includes limited and repetitive patterns of behavior.
Learning disabilities	**Learning disabilities interfere with learning basic skills such as reading, writing and/or math. They can also interfere with higher level skills such as organization, time planning, abstract reasoning, memory and attention. Examples include dyscalculia (math), dysgraphia (writing), and dyslexia (reading),**
Other developmental problem (describe):	Examples include **OCD, Social Anxiety Disorder, Phobias, PTSD, Bulimia, Anorexia Nervosa, Binge Eating, etc.** Some developmental disorders, such as **Fragile X syndrome** and **fetal alcohol syndrome**, also typically result in **low IQ (an exclusion criterion for this study**). (Do not score mental disorders that are detected by the SCID here.)
Eczema	Eczema (**atopic dermatitis)** is an itchy rash. Atopic dermatitis is long lasting (chronic) and tends to flare periodically. It may be accompanied by asthma or hay fever.
Psoriasis	Psoriasis is a skin disease that causes red, itchy scaly patches, most commonly on the knees, elbows, trunk and scalp.
SLE (lupus)	**Lupus is an autoimmune disease that may cause psychosis**. Symptoms include inflammation in joints, skin, kidneys, blood cells, brain, heart and lungs. Lupus is often associated with a facial rash that resembles the wings of a butterfly unfolding across both cheeks.
Other skin rash (describe):	Do not code common contact rashes (e.g. poison ivy) here. If the study participant currently has a skin rash record this on the "Current Health Status" form. **Scarlet fever** may develop with a strep infection and is a bright red rash that covers most of the body. Scarlet fever is almost always accompanied by a sore throat and a high fever.
Cardiovascular disease/heart disease	Children may be born with various congenital heart defects. Code **Multisystem inflammatory syndrome (MIS-C)** or **Kawasaki disease** under "other".
Coronary artery disease	Coronary artery disease is a heart condition, where plagues build up inside the arteries of the heart. While unusual in adolescents and young adults, it may be associated with obesity or with **familial hypercholesterolemia** (a genetic disorder) that affects the way the body processes cholesterol.
Myocarditis	Myocarditis is an inflammation of the heart muscle (myocardium) that may reduce the ability of the heart to pump blood and lead to arrhythmias. A viral infection usually causes myocarditis, but it can result from a reaction to a drug or be part of a more general inflammatory condition.
Other heart condition (describe):	**Pericarditis** is swelling and irritation of the thin, saclike tissue surrounding the heart (pericardium). Pericarditis often causes sharp chest pain. There are a variety of structural heart problems and arrythmias.
Celiac disease	Celiac disease, (**celiac sprue** or **gluten-sensitive enteropathy)**, is an immune reaction to eating gluten, a protein found in wheat, barley and rye that affects the small intestine. The intestinal damage often causes diarrhea, fatigue, weight loss, bloating and anemia, and can lead to serious complications. **Non-celiac gluten sensitivity** is coded under "other".
Crohn's disease	Crohn's disease is a autoimmune inflammatory disease of the gastrointestinal tract. Symptoms include diarrhea and constipation. Crohn's disease is chronic and may appear and disappear at various times.
Gastritis	Gastritis is a general term for a group of conditions involving inflammation of the lining of the stomach. The inflammation of gastritis is most often the result of infection with the same bacterium that causes most **stomach ulcers**.

Irritable bowel syndrome (IBS)	Irritable bowel syndrome (IBS) is a common disorder of unknow etiology that affects the large intestine. Signs and symptoms include cramping, abdominal pain, bloating, gas, and diarrhea or constipation, or both.
Ulcerative colitis	**Ulcerative colitis is an autoimmune disorder** the large intestine, in which the lining of the colon becomes inflamed and develops tiny open sores, or ulcers.
Other GI problem (describe):	Examples include gastroesophageal reflux disease (GERD) and lactose intolerance
Migraine headaches	A migraine headache involves throbbing pain or a pulsing sensation, usually on one side of the head. It's often accompanied by nausea, vomiting, and extreme sensitivity to light and sound. Migraine attacks can last for hours to days, and the pain can be so severe that it interferes with your daily activities. Some people may have **aura's** that often happen right before the headache. **An aura can include visual disturbances, such as flashes of light or blind spots, or other disturbances, such as tingling on one side of the face or in an arm or leg and difficulty speaking.**
Multiple sclerosis	Multiple sclerosis (**MS**) is an autoimmune disorder of the brain. In MS, the immune system attacks the protective sheath (myelin) that covers nerve fibers and causes communication problems between your brain and the rest of your body. Eventually, the disease can cause permanent damage or deterioration of the nerves. Signs and symptoms of MS vary widely and depend on the amount of nerve damage and which nerves are affected. Some people with severe MS may lose the ability to walk independently or at all, while others may experience long periods of remission without any new symptoms. **Rarely MS is associated with psychosis symptoms.**
Seizures	A seizure is a sudden, uncontrolled electrical disturbance in the brain. It can cause changes in behavior, movements or feelings, and in levels of consciousness. Having two or more seizures at least 24 hours apart that aren't brought on by an identifiable cause is generally considered to be **epilepsy**. There are many types of seizures, which range in symptoms and severity. Seizure types vary by where in the brain they begin and how far they spread**. Absence seizures** (also petit male seizures) involves a person's brain "blanking out", appearing to star off into space for a few seconds or up to a minute. During this time the person isn't aware of their surroundings. **Absence seizures can be mistaken for disorganized thought process/thought blocking.** **History a single, febrile seizure in early childhood is not coded here**.
Tension headaches	A tension headache is generally a diffuse, mild to moderate pain, often described as feeling like a tight band around your head.
Concussion/traumatic brain injury	Concussions are usually caused by a blow or violent shaking of the head (e.g. car accident, sports injury, falls). Some concussions cause loss of consciousness, but most do not. Post-concussion symptoms include headaches and problems with concentration, memory, balance and coordination. **Severe concussions are exclusionary for this study.**
Other neurological problem (describe):	Examples include **cerebral palsy, muscular dystrophy, optic neuritis (Devic's syndrome).**
Hepatitis A	Hepatitis A is a highly contagious liver infection. The virus is spread from contaminated food, water, or surfaces. Hepatitis A is not chronic.
Hepatitis B	Hepatitis B is a liver infection that may become chronic with increased risk of liver damage. The virus spreads by contaminated blood.
Hepatitis C	Hepatitis C is a liver infection that may become chronic with increased risk of liver damage. The virus spreads through contaminated blood.
*HIV/AIDS	Acquired immunodeficiency syndrome (AIDS) is a chronic, potentially life-threatening condition caused by the human immunodeficiency virus (HIV). **HIV can infect the brain and cause cognitive impairments.**
HSV-1 ("oral herpes" or "cold sores")	HSV causes cold sores. After the initial infection, the virus lies dormant but then reactivates periodically.

HSV-2 ("genital herpes")	HSV causes cold sores. After the initial infection, the virus lies dormant but then reactivates periodically.
Mononucleosis	Infectious mononucleosis (**mono, kissing disease**) caused by the Epstein-Barr virus. Spread by saliva, symptoms include fever and a sore throat, as well as fatigue, and enlarged lymph nodes and spleen that may last several weeks.
Other viral infection (describe):	Record any significant viral infections that could become chronic, such as **Lyme disease, or Brucellosis (cat scratch fever)**
"How about cancer?"	The most common types of cancer diagnosed in children and adolescents include **leukemias, lymphomas, and brain tumors.**
"How about sleep apnea?"	Sleep apnea is when breathing repeatedly stops and re-starts during sleep. It often occurs in persons with obesity.
"Any signs of gingivitis or periodontitis?"	Gingivitis/periodontitis are chronic inflammation in the mouth typically related to bacterial overgrowth. Symptoms include swollen, tender, or receding gums, or gums that bleed easily.
"How about polycystic ovary syndrome (PCOS)?"	Polycystic ovary syndrome (PCOS) is a hormonal disorder common among women of reproductive age. Females with PCOS may have infrequent or prolonged menstrual periods or excess male hormone (androgen) levels. The ovaries may develop numerous small collections of fluid (follicles) and fail to regularly release eggs. Signs and symptoms of PCOS often develop around the time of the first menstrual period during puberty. Sometimes PCOS develops later, for example, in response to substantial weight gain.
"Anything else?" (describe):	**Multisystem inflammatory syndrome, (MIS-C) or Kawasaki disease** involves swelling (inflammation) in the walls of medium-sized arteries throughout the body. It primarily affects children. The inflammation tends to affect the coronary arteries, which supply blood to the heart muscle. Kawasaki disease is sometimes called **mucocutaneous lymph node syndrome.** There is an association of MIS-C as a post-Covid 19 infection syndrome. **DiGeorge syndrome (also 22q11 deletion syndrome, velocardiofacial syndrome),** is caused when a small part of chromosome 22 is missing. This deletion results in the poor development of several body systems. **About 60% of persons with this syndrome develop a psychotic disorder.**

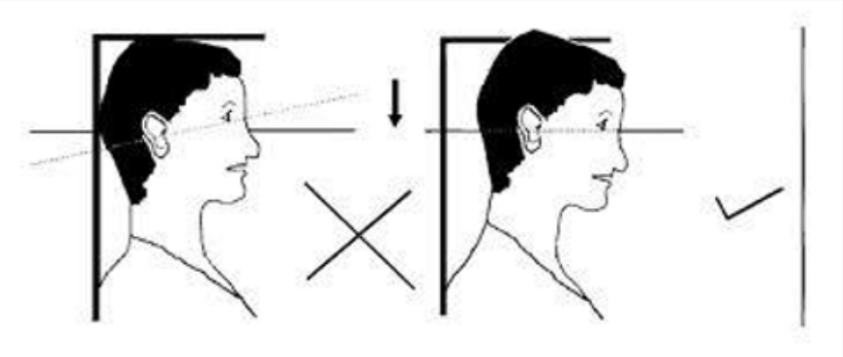
D. Current Health Status

Current Health Status Form	
Study assessment date and time (24-hour clock)	_____/____/____ _____:_____ YYYY / MM / DD HH : MM

Height, Weight, Heart Rate, Blood Pressure, and Body Temperature.

Height Measurement Procedure:

- **Ask the study participant to stand with back to wall, with shoulder blades and bottom touching wall.**
- **Ask the study participant to "stand up straight and look straight ahead".**
- **An imaginary line drawn from bottom of eye socket to top of ear canal should be parallel to floor. The stadiometer bar is placed on the top of the head, compressing hair as much as possible.**
- **Record the height, noting whether measurements are taken in inches or centimeters.**



	Measurement	Units of Measurement
Height		☐ Centimeters ☐ Inches

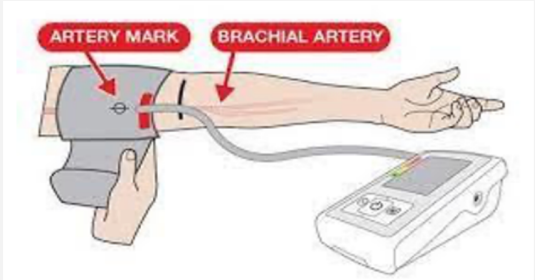
Weight Measurement Procedure:

- **Ask the study participant to remove shoes, jackets, sweaters, and belt.**
- **Have study participant empty his/her pockets.**
- **Have study participant stand on scale.**
- **Record weight, noting whether measurements are taken in pounds or kilograms.**

	Measurement	Units of Measurement
Weight		☐ Kilograms ☐ Pounds

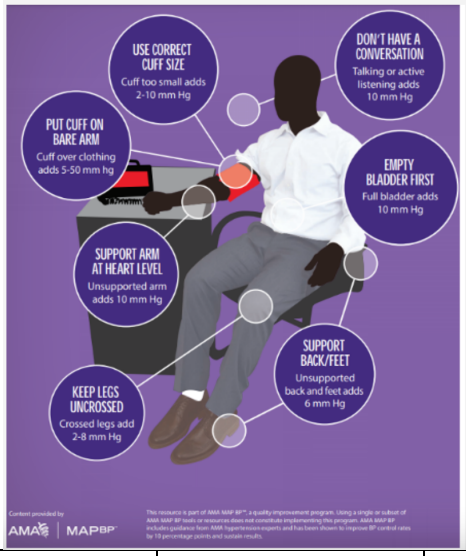
Placing the blood pressure cuff on the study participant's arm:

- **Make sure the cuff is the correct size for the study participant's arm size. You know the size is correct when the markings of the cuff intersect when wrapped around the study participant's upper arm.**



Procedures to ensure accurate blood pressure measurement:

- **Have the study participant sit, with both feet flat on the ground (legs not crossed) and back supported.**
- **The upper arm should be bare.**
- **The arm should be supported so that the upper arm is at the level of the person's heart.**
- **Make sure the cuff is entirely deflated. Wrap the cuff around the arm, lining up the artery mark over the brachial artery as shown at right.**
- **Instruct the study participant to sit quietly while their blood pressure is being taken. Start the blood pressure machine.**
- **Don't have a conversation with the study participant while the blood pressure measurement is taking place!**
- **It is normal for blood pressure to be somewhat elevated after physical activity, with anxiety, or if the above protocol is not followed. If the blood pressure is above 120/80, check to make sure the protocol is being followed, and re-check the blood pressure after the person has been seated for five minutes.**
- **Record the blood pressure (higher number = systolic, lower number = diastolic) and heart rate. If the blood pressure was repeated, record the second blood pressure.**



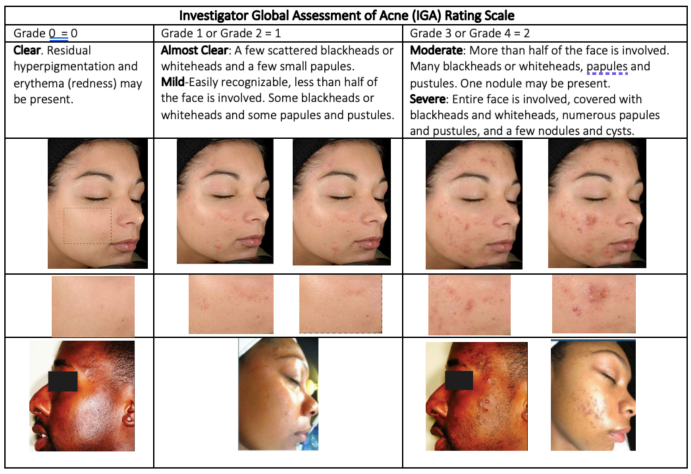
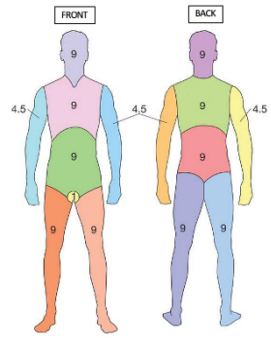
Heart Rate		bpm
Systolic blood pressure		mmHg
Diastolic blood pressure		mmHg
Body Temperature		☐ Celsius ☐ Fahrenheit

Sleep and wake times, last meal and/or beverage (prior to blood draw), tobacco and/or marijuana use (prior to blood draw), and menstrual period.

	Date (YYYY/MM/DD)	**Time** (24-hr)

Date and **time** the study participant went to bed the night before the assessment		___/____/_____	_____:_____
Date and **time** the study participant woke-up.		___/____/_____	_____:_____
Date and **time** the study participant last ate or drank anything with calories (water does not count) **prior to blood draw**. *(This information is used to determine how long the study participant was fasting prior to the blood draw.)*		___/____/_____	_____:_____
Date and **time** the study participant last used tobacco **prior to blood draw**.	Non-user	___/____/_____	_____:_____
Date and **time** the study participant last used marijuana **prior to blood draw**.	Non-user	___/____/_____	_____:_____
Date study participant began last menstrual period.	Not Applicable	___/____/_____	

Acute inflammatory conditions.

Have you been sick in the **past month**? *(Include a cold, flu, sore throat, ear infection, GI bug, etc.)*	☐ 0, Not sick in past month ☐ 1, Mildly sick: just annoying ☐ 2, Moderately sick: sick at home but could still function ☐ 3, Severely sick: in bed, couldn't do much of anything	
If mildly, moderately, or severely sick in the past month, what was the last **date** (YYYY/MM/DD) you had any symptoms? *(Estimate if needed)*	_____/_____/_____	N/A
IGA (acne severity rating) 	☐ 0, **Clear**: Residual hyperpigmentation and erythema (redness) may be present. ☐ 1, **Almost Clear**: A few scattered blackheads or whiteheads and a few small papules. OR **Mild**-Easily recognizable, less than half of the face is involved. Some blackheads or whiteheads and some papules and pustules. ☐ 2**, Moderate**: More than half of the face is involved. Many blackheads or whiteheads, papules and pustules. One nodule may be present. ☐ **3, Severe**: Entire face is involved, covered with blackheads and whiteheads, numerous papules and pustules, and a few nodules and cysts.	
Instructions: Ask study participant if they have a sunburn. If yes, ask the study participant to indicate what body areas are involved. Rate the percent of the body involved using the following scale. For example, if a study participant's arms and lower legs were sunburned in the back but not the front, the score would be 4.5+4.5+9+9=27. 	_____%	

Physical activity.

Minutes **today** (after midnight) spent doing physical activity that was intense enough to make the study participant breathe a bit hard or work up a sweat:	_____ minutes

Review and update the Medication Log.		
With the study participant's Medication Log in hand, review OTC and prescription medications currently in use and frequency of use. If the study participant reports any changes or new medications, make needed changes on the Medication Log. ***Make sure to ask specifically about use of medications in each of the following categories: anti-inflammatories, pain medicine, allergy and/or cold medicine, diet pills, vitamins or other supplements, and antibiotics or steroids.***		
Medication use reviewed and data recorded on Medication Log?	Yes No	
COVID-19.		
Have you been suspected of having COVID-19?	☐ 0, **No** symptoms or signs ☐ 1, **Maybe,** had possible symptoms, but no diagnosis and no test ☐ 2, **Maybe,** received a medical diagnosis but no test ☐ 3, **Yes,** had a positive test	
If yes, approximately when was the first time that you were diagnosed with COVID-19 or when did your possible symptoms begin?	_____/_____/_____	N/A
If yes, when you were sick with COVID-19 (diagnosed or suspected), approximately how many days were you sick?		N/A
If yes, have you been hospitalized because of COVID-19?	☐ Yes ☐ No	N/A
Have you received a COVID-19 vaccine (may choose multiple)?	☐ 0, No vaccine ☐ 1, **Yes,** Pfizer-BioNTech vaccine ☐ 2, **Yes,** Moderna vaccine ☐ 3, **Yes** Johnson & Johnson vaccine ☐ 4, **Yes,** AstraZeneca vaccine ☐ 5, **Yes,** Novavax vaccine ☐ 99, **Yes,** Other or unknown (specify):	
If yes, how many vaccine doses have you received? **include booster dose(s)*	_____	N/A
When did you receive the first dose of the vaccine *(if applicable)?*	_____/_____/_____	N/A
When did you last receive a booster dose of the vaccine *(if applicable)?*	_____/_____/_____	N/A

Preparing study participant for the blood draw.

The Applied Tension Technique to Prevent Vasovagal Syncope

1. Done while seated, a few minutes prior to blood draw, and during the blood draw.
2. Tense muscles in legs, arm, and upper body. Hold for 10-15 seconds, or until you feel warmth rising in your face. (Do not tense the arm that is used for the blood draw during the blood draw.)
3. Release tension for 20-30 seconds.
4. Repeat 5 times prior to the blood draw, and during the blood draw.

What strategies did you use to prepare the study participant for the blood draw?	☐ Asked the study participant about their previous experiences with having their blood drawn, including if they have ever felt light-headed or fainted. ☐ Asked the study participant if they had any questions about the blood draw. ☐ Responded to the participant's question(s) about the blood draw with information. Answers to common questions include: a) the amount of blood taken is small, about 3 tablespoons (not enough make any difference in how they feel), b) the main risks from a blood draw are bruising, that the needle might hurt, and that some people may become light-headed or faint, c) feeling light-headed or fainting a reflex response (the vasovagal reflex) and is not due to the amount of blood that is taken. ☐ Explained and had the study participant practice the Applied Tension Technique as a tool to prevent feeling light-headed or fainting (see above). ☐ Distracted the study participant during the blood draw (for example, by engaging in conversation). ☐ Informed the phlebotomist of previous issues with a blood draw, such as a history of feeling light-headed or fainting. ☐ Other ☐ None

E. Vital Signs Clinical Review Form

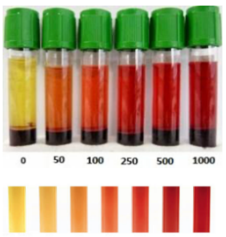
Vital Signs Clinical Review Form (completed by Study PI or Designate)	
Study participant ID	
Reviewer name	
Date of review	
If the study participant's blood pressure and temperature are normal the following message will be displayed: Blood pressure and temperature are within normal ranges.	
For any values outside of normal ranges the following is displayed:	
Systolic blood pressure value::	
Systolic blood pressure value clinically significant?	☐ Yes ☐ No
Response to clinically significant finding:	☐ notified study participant ☐ unable to notify study participant ☐ recommended study participant follow-up with primary care provider ☐ recommended study participant follow-up with emergency services/urgent care for immediate treatment ☐ other
If unable to reach, please describe efforts to reach study participant:	
If other, please describe:	
Diastolic blood pressure value:	
Diastolic blood pressure value clinically significant?	☐ Yes ☐ No
Response to clinically significant finding:	☐ notified study participant ☐ unable to notify study participant ☐ recommended study participant follow-up with primary care provider ☐ recommended study participant follow-up with emergency services/urgent care for immediate treatment ☐ other
If unable to reach, please describe efforts to reach study participant:	
If other, please describe:	
Body temperature value:	
Body temperature value clinically significant?	☐ Yes ☐ No
Response to clinically significant finding:	☐ notified study participant ☐ unable to notify study participant ☐ recommended study participant follow-up with primary care provider ☐ recommended study participant follow-up with emergency services/urgent care for immediate treatment ☐ other
If unable to reach, please describe efforts to reach study participant:	
If other, please describe:	

F. Blood Sample Preanalytic Quality Assurance Form

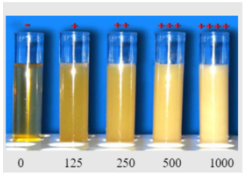
Blood Sample Preanalytic Quality Assurance Form	
Blood sample collection date and time (24-hour clock)	_____/____/____ _____:_____ YYYY / MM / DD HH : MM
Fidelity to Sample Collection Protocols:	
Record any blood collection issues or protocol deviations	
Was the 3mL EDTA tube sent to a central laboratory for a CBC w/Differential?	Yes No
Sample Receipt by Processing Laboratory:	
Date and time blood samples received in lab	_____/____/____ _____:_____ YYYY / MM / DD HH : MM
Centrifuge Details:	
Temperature of centrifuge	_____
Centrifuge temperature units	°C °F
Sample Aliquots:	

ProNET Only:

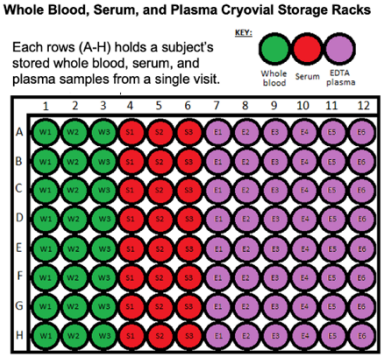
Hemolysis Rating Scale Guide



Lipemia Rating Scale Guide



Whole Blood, Serum, and Plasma Cryovial Storage Racks

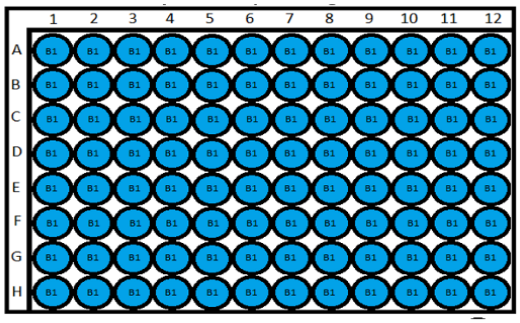


Sample Type	Cryotube Barcode	Volume (mL) (target volume is 1 mL)	Hemolysis (from 0-1000, estimate based on Hemolysis Rating Scale Guide)	Lipemia (from 0-1000 mg/dL, estimate based on Lipemia Rating Scale Gude)	Cryotube Rack Barcode	Position in Cryotube Rack	Date and time stored in -80°C Freezer (24-hr clock)
Whole Blood (W1)							_____/____/__ _ __:__ YYYY /MM/DD HH:MM
Whole Blood (W2)							
Whole Blood (W3)							
Serum (S1)			0 20 50 100 250 500 1000	0 125 250 500 1000			_____/____/__ _ __:__ YYYY /MM/DD HH:MM
Serum (S2)							
Serum (S3)							
EDTA Plasma (E1)			0 20 50	0			

			100 250 500 1000	125 250 500 1000			
EDTA Plasma (E2)			0 20 50 100 250 500 1000				
EDTA Plasma (E3)			0 20 50 100 250 500 1000				_____/____/__ _ __:__ YYYY /MM/DD HH:MM
EDTA Plasma (E4)			0 20 50 100 250 500 1000				
EDTA Plasma (E5)			0 20 50 100 250 500 1000				
EDTA Plasma (E6)			0 20 50 100 250 500 1000				

Buffy Coat Cryovial Storage Rack

The rack stores the buffy coat cryovials of 96 subjects.



Buffy Coat (B1)							_____/____/__ _ __:__ YYYY /MM/DD HH:MM

Fidelity to Blood Processing and Storage Protocols:	
Cryotube Rack Description *(Description should include study name, sample type (if samples are stored in separate racks), time-point/visit, rack number (incremental digits i.e., 1,2 3)).*	
Processing time (minutes) whole blood. (*Automatically calculated in electronic data base as time placed in freezer minus time of blood draw, in minutes, flag for checking if greater than 90 minutes or less than 30 minutes.*)	
Processing time (minutes) serum.	

(*Automatically calculated in electronic data base as time placed in freezer minus time of blood draw, in minutes, flag for checking if greater than 90 minutes or less than 30 minutes.*)	
Processing time (minutes) plasma. (*Automatically calculated in electronic data base as time placed in freezer minus time of blood draw, in minutes, flag for checking if greater than 90 minutes or less than 30 minutes.*)	
Processing time (minutes) buffy coat. (*Automatically calculated in electronic data base as time placed in freezer minus time of blood draw, in minutes, flag for checking if greater than 90 minutes or less than 30 minutes.*)	
Storage Freezer ID (should match ID on Freezer Temperature Monitoring Log)	
Cryotube Rack Description: [Description should include study name, time-point/visit, rack number (incremental digits i.e. 1,2,3), other relevant lab-specific details]	
Record any blood processing issues or protocol deviations	

G. Daily Activity and Saliva Collection Form

Daily Activity and Saliva Sample Collection Form				
Report consumption of food and drinks.				
Have any of the following types of food or drinks been consumed **since 6PM (18:00) yesterday**?				
Foods or drinks containing caffeine or xanthenes *(i.e. coffee, caffeinated tea, chocolate, cola products, energy drinks, etc.)*	Yes	No		
Alcohol	Yes	No		
Dairy products	Yes	No		
Review and update the Medication Log.				
With the study participant's Medication Log in hand, review OTC and prescription medications currently in use and frequency of use. If the study participant reports any changes or new medications, make needed changes on the Medication Log. ***Make sure to ask specifically about use of medications in each of the following categories: anti-inflammatories, pain medicine, allergy and/or cold medicine, diet pills, vitamins or other supplements, and antibiotics or steroids.***				
Medication use reviewed and data recorded on Medication Log?	Yes	No		
Report recent activity.				
Has study participant used tobacco today?	Yes	No		
Has study participant used marijuana today?	Yes	No		
Has study participant done any aerobic or weight exercises today?	Yes	No		
Has study participant had dental work performed within 24 hours of sample collection?	Yes	No		
Has study participant had a major meal within 60 minutes of sample collection?	Yes	No		
Did study participant brush/floss his or her teeth within 45 minutes of sample collection?	Yes	No		
	Date (YYYY/MM/DD)	Time (24-hour)		
Date and **time** the study participant went to bed the night before the assessment	___/___/_____	_____:_____		
Date and **time** the study participant woke up the day of the assessment	___/___/_____	_____:_____		
Hours study participant slept (calculated by electronic data base) *(flag if hours slept is a negative number)*				
Saliva sample collection details.				

Please have participant review this 4 minute training video prior to their collection of saliva samples.

Date (YYYY/MM/DD) of saliva sample collection	___/___/_____
Were all saliva samples placed on ice immediately after collection and kept on ice until stored?	Yes No

	Collection Time (24-hour clock)	Did study participant drink within 10 min prior to collection?	Sample	Cryotube Barcode	Volume (mL)	Cryotube Rack Barcode	Position in Cryotube Rack	Time stored in -20°C or -80°C °freezer (24-hour clock)
1	_____:_____	Yes No	Sample 1A					___:___
			Sample 1B					
2	_____:_____	Yes No	Sample 2A					___:___
			Sample 2B					
3	_____:_____	Yes No	Sample 3A					___:___
			Sample 3B					

Cryotube Rack Description *(Description should include study name, sample type (if samples are stored in separate racks), time-point/visit, rack number (incremental digits i.e., 1,2 3)).*	_____:_____	
Storage Freezer ID		
Record any saliva collection issues or protocol deviations.		

H. CBC with Differential Reporting Form

CBC with Differential Form

Instructions:

- Ensure that the study participant's results are entered using the measurement unit specified on the data entry form. Different labs have different conventions, especially when reporting cell counts.
- Examples include:
  - Some labs report the actual number of cells, others report the number of cells using "scientific notation" (power of 10): 1000 = 10^3 = 10^3
  - Some labs report the number of cells per liter (L) of blood, others per mililiter (mL), others per microliter (uL): 1L = 1000 mL = 1,000, 000 uL
  - The majority of “white blood cells” (WBCs) are neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Most labs report the absolute number of the different WBCs and their percentage out of all the WBCs. A few labs only report the percentages; in this case you need to derive the absolute number of the different cell types (by multiplying the absolute number of WBCs by the proportion of the cell type).

If you are unsure how to enter the CBC with differential data into the study data base, contact us for help.

Measurement Name	**Result**	Result Units *(please ensure that results are provided in the indicated units)*	Is the result value outside of reference range provided by the report?	For values outside of the reference range, enter the report's reference range **lower value** (in the units specified on this form).	For values outside of the reference range, enter the report's reference range **upper value** (in the units specified on this form).
Red Blood Cell (RBC)		x 10^{12}/L	☐ 1 Yes ☐ 0 No		
Hematocrit (Hct)		%	☐ 1 Yes ☐ 0 No		
Hemoglobin (Hgb)		g/L	☐ 1 Yes ☐ 0 No		
Mean Corpuscular Volume (MCV)		fL	☐ 1 Yes ☐ 0 No		
Mean Corpuscular Hemoglobin (MCH)		pg	☐ 1 Yes ☐ 0 No		
Mean Corpuscular Hemoglobin Concentration (MCHC)		g/L	☐ 1 Yes ☐ 0 No		

Platelet Count		x 10^9/L	☐$_1$ Yes ☐$_0$ No		
Mean Platelet Volume (MPV)		fL	☐$_1$ Yes ☐$_0$ No		
White Blood Cell Count (WBC)		x 10^9/L	☐$_1$ Yes ☐$_0$ No		
Absolute Neutrophils (Neut)		x 10^9/L	☐$_1$ Yes ☐$_0$ No		
Absolute Lymphocytes (Lymph)		x 10^9/L	☐$_1$ Yes ☐$_0$ No		
Absolute Monocytes (Mono)		x 10^9/L	☐$_1$ Yes ☐$_0$ No		
Absolute Eosinophils (Eos)		x 10^9/L	☐$_1$ Yes ☐$_0$ No		
Absolute Basophils (Baso)		x 10^9/L	☐$_1$ Yes ☐$_0$ No		
Sum of absolute neutrophils, lymphocytes, monocytes, basophils, and eosinophils	_____ (Calculated by electronic data base. Flag if the sum is not equal to (+/- 0.1) the WBC count)				

I. CBC with Differential Clinical Review Form

CBC with Differential Clinical Review Form (completed by Study PI or Designate)	
Study participant ID	
Reviewer name	
Date of review	
If no values from CBC with Differential are outside of reference range: No values from the CBC with differential lab results were outside of the normal reference range.	
For any values that are outside of the normal reference range the reported value, reference range, and units are reported:	
RBC count result: Reference range lower limit: Reference range upper limit: Units: 10^12/L equivalent to 10^6/uL	
RBC count value clinically significant?	☐ Yes ☐ No
Response to clinically significant finding:	☐ notified study participant ☐ unable to notify study participant ☐ recommended study participant follow-up with primary care provider ☐ recommended study participant follow-up with emergency services/urgent care for immediate treatment ☐ other
If unable to reach, please describe efforts to reach study participant:	
If other, please describe:	
Hematocrit (Hct) Result: Reference range lower limit: Reference range upper limit: units: % (percent)	
Hematocrit value clinically significant?	☐ Yes ☐ No
Response to clinically significant finding:	☐ notified study participant ☐ unable to notify study participant ☐ recommended study participant follow-up with primary care provider ☐ recommended study participant follow-up with emergency services/urgent care for immediate treatment ☐ other
If unable to reach, please describe efforts to reach study participant:	
If other, please describe:	
Hemoglobin Result: Reference range lower limit: Reference range upper limit: units: g/L (grams/liter)	
Hematocrit value clinically significant?	☐ Yes ☐ No
Response to clinically significant finding:	☐ notified study participant ☐ unable to notify study participant ☐ recommended study participant follow-up with primary care provider ☐ recommended study participant follow-up with emergency services/urgent care for immediate treatment

	☐ other
If unable to reach, please describe efforts to reach study participant:	
If other, please describe:	
Mean Corpuscular Volume (MCV) Result: Reference range lower limit: Reference range upper limit: units: fL (femtoliter)	
MCV value clinically significant?	☐ Yes ☐ No
Response to clinically significant finding:	☐ notified study participant ☐ unable to notify study participant ☐ recommended study participant follow-up with primary care provider ☐ recommended study participant follow-up with emergency services/urgent care for immediate treatment ☐ other
If unable to reach, please describe efforts to reach study participant:	
If other, please describe:	
Mean Corpuscular Hemoglobin (MCH) Result: Reference range lower limit: Reference range upper limit: units: pg (picogram)	
MCH value clinically significant?	☐ Yes ☐ No
Response to clinically significant finding:	☐ notified study participant ☐ unable to notify study participant ☐ recommended study participant follow-up with primary care provider ☐ recommended study participant follow-up with emergency services/urgent care for immediate treatment ☐ other
If unable to reach, please describe efforts to reach study participant:	
If other, please describe:	
Mean Corpuscular Hemoglobin Concentration (MCHC): Reference range lower limit: Reference range upper limit: units: g/L (grams/liter)	
MCHC value clinically significant?	☐ Yes ☐ No
Response to clinically significant finding:	☐ notified study participant ☐ unable to notify study participant ☐ recommended study participant follow-up with primary care provider ☐ recommended study participant follow-up with emergency services/urgent care for immediate treatment ☐ other
If unable to reach, please describe efforts to reach study participant:	
If other, please describe:	
Platelet Count Result: Reference range lower limit:	

Reference range upper limit: units: 10^9/L equivalent to 10^3/uL	
WBC count value clinically significant?	☐ Yes ☐ No
Response to clinically significant finding:	☐ notified study participant ☐ unable to notify study participant ☐ recommended study participant follow-up with primary care provider ☐ recommended study participant follow-up with emergency services/urgent care for immediate treatment ☐ other
If unable to reach, please describe efforts to reach study participant:	
If other, please describe:	
White Blood Cell Count (WBC) Results: Reference range lower limit: Reference range upper limit: units: 10^9/L equivalent to 10^3/uL	
MCV value clinically significant?	☐ Yes ☐ No
Response to clinically significant finding:	☐ notified study participant ☐ unable to notify study participant ☐ recommended study participant follow-up with primary care provider ☐ recommended study participant follow-up with emergency services/urgent care for immediate treatment ☐ other
If unable to reach, please describe efforts to reach study participant:	
If other, please describe:	
Neutrophil Count Result: Reference range lower limit: Reference range upper limit: units: 10^9/L equivalent to 10^3/uL	
Neutrophil count value clinically significant?	☐ Yes ☐ No
Response to clinically significant finding:	☐ notified study participant ☐ unable to notify study participant ☐ recommended study participant follow-up with primary care provider ☐ recommended study participant follow-up with emergency services/urgent care for immediate treatment ☐ other
If unable to reach, please describe efforts to reach study participant:	
If other, please describe:	
Lymphocyte Count Result: Reference range lower limit: Reference range upper limit: units: 10^9/L equivalent to 10^3/uL	
Lymphocyte Count value	☐ Yes ☐ No

clinically significant?	
Response to clinically significant finding:	☐ notified study participant ☐ unable to notify study participant ☐ recommended study participant follow-up with primary care provider ☐ recommended study participant follow-up with emergency services/urgent care for immediate treatment ☐ other
If unable to reach, please describe efforts to reach study participant:	
If other, please describe:	
Monocyte Count Result: Reference range lower limit: Reference range upper limit: units: 10^9/L equivalent to 10^3/uL	
Monocyte Count value clinically significant?	☐ Yes ☐ No
Response to clinically significant finding:	☐ notified study participant ☐ unable to notify study participant ☐ recommended study participant follow-up with primary care provider ☐ recommended study participant follow-up with emergency services/urgent care for immediate treatment ☐ other
If unable to reach, please describe efforts to reach study participant:	
If other, please describe:	
Eosinophil Count Result: Reference range lower limit: Reference range upper limit: units: 10^9/L equivalent to 10^3/uL	
Eosinophil Count value clinically significant?	☐ Yes ☐ No
Response to clinically significant finding:	☐ notified study participant ☐ unable to notify study participant ☐ recommended study participant follow-up with primary care provider ☐ recommended study participant follow-up with emergency services/urgent care for immediate treatment ☐ other
If unable to reach, please describe efforts to reach study participant:	
If other, please describe:	
Basophil Count Result: Reference range lower limit: Reference range upper limit: units: 10^9/L equivalent to 10^3/uL	
Basophil Count value clinically significant?	☐ Yes ☐ No
Response to clinically	☐ notified study participant ☐ unable to notify study participant ☐ recommended study participant follow-up with primary care provider

significant finding:	☐ recommended study participant follow-up with emergency services/urgent care for immediate treatment ☐ other
If unable to reach, please describe efforts to reach study participant:	
If other, please describe:	
Response to clinically significant finding:	☐ notified study participant ☐ unable to notify study participant ☐ recommended study participant follow-up with primary care provider ☐ recommended study participant follow-up with emergency services/urgent care for immediate treatment ☐ other
If unable to reach, please describe efforts to reach study participant:	
If other, please describe:	

J. Freezer Temperature Monitoring Log

Use this log if your site does not have a digital monitoring method. A separate log must be kept for each freezer. Min/max temperature should be recorded at least once per week. If extended temperature monitoring data is available, it should be printed and kept with site documentation.

Record all temperature excursions outside of the allowable range on the Freezer Temperature Excursion Log.

Freezer temperature: _____ Allowed temperature excursion range: _____

Freezer location: _____ Freezer name (if available): _____

Monitoring method (Serial No. and Calibration Expiry Date if applicable): _____

Items stored: _____

Dates (mm/dd/yy)		Temperature		Initials	Dates (mm/dd/yy)		Temperatures		Initials
Start	End	Min	Max		Start	End	Min	Max	

K. Freezer Temperature Excursion Log

PRESCIENT

Please contact your team lead or Dr. Phassouliotis for further instructions.

ProNET

The ProNET Genetics and Fluids Core has created a template to track freezer excursions. When you're alerted to an excursion for any of your freezers, please fill out the Freezer Temperature Excursion Log template found in the shared Google Drive folder. Row 3 has been filled with example data (please overwrite this row when filling out your excursions, but careful not to delete the formula in D3!).

After the spreadsheet has been filled out, please upload to your YaleBox Regulatory folder and email Rachel Bleggi (rachel_bleggi@med.unc.edu), Dr. Perkins (diana_perkins@med.unc.edu), Susan Ray (sray@northwell.edu), and Priya Matneja (pmatneja@northwell.edu) to alert us to the excursion.

This only needs to be uploaded on an as-needed basis (not monthly!). Site coordinators are ultimately responsible for logging this information, but please share this spreadsheet with any lab managers so they can be aware of the requested information as well. If you have any questions regarding this spreadsheet or freezer excursions, please reach out to the G&F Core.