UCSD-IU ALS Metabolomics Study
IRB# 2009735565

Phlebotomy and Sample Processing Orders
January 7, 2021

Phlebotomy

1. 4 ml of venous blood drawn into a lithium-heparin vacutainer tube (green-top tube; without gel separator [use of a gel separator tube is not acceptable]; 13 x 75 mm; BD #367884; not sodium-heparin)
    a. Gently invert x 10 to mix
    b. Keep at room temperature until plasma separation. Do not refrigerate. Refrigeration can activate platelets.
    c. Separate plasma and whole cells by centrifugation within 1 hour of whole blood collection
        i. Plasma is for metabolomics
        ii. Whole cells are for epigenetics

2. 2.5 ml of venous blood drawn into a PAXGene RNA tube kept in an upright position for at least 10 seconds for full collection during venipuncture (16 x 100 mm with 6.9 ml of preservative; BD #762165). See attached "PAXGene tube blood collection guide"
    a. Gently invert x 10 to mix
    b. Be sure the PAXGene tube is labeled with: patient name, date, time, and "Naviaux ALS Study"
    c. The PAXGene tubes are for microRNA analysis

Sample Processing

1. Green-top tubes for plasma and whole cells
    a. Centrifuge for 10 minutes at 900 g (typically about 1200-1500 rpm for most clinical centrifuges). Do not centrifuge for longer than 10 minutes.
    b. Using a sterile transfer pipet or Pipetman, transfer and divide the plasma into two, (not one) 1.8 ml Nunc cryotubes (Thermo, #375418) labeled "Heparin-plasma".
    c. Keep the remaining red and white cell fraction, mix and transfer to cryotubes labeled "Heparin-cells"
    d. Label the tubes with: patient name, date, time, and "Naviaux ALS Study"

2. PAXGene RNA tubes
    a. No further processing is required

Sample Storage

1. Labeled cryotubes (separate tubes for plasma and whole cells)
    a. Heparin-plasma: store at -70˚ to -80˚C until the total set of 120 patient samples (30 subjects x 4 samples/subject x 2 tubes/sample time = 240 cryotubes) has been collected. -20˚C storage is not acceptable.
    b. Heparin-cells: same as above

2. PAXGene RNA tubes
    a. Store at -70˚ to -80˚C until the total set of 120 PAXGene tubes (30 subjects x 4 samples/subject) has been collected

Sample Shipment

1. When the completed set of samples is ready for shipment, please email Dr. Naviaux at rnaviaux@health.ucsd.edu before shipping to alert Naviaux Lab personnel at UCSD and to coordinate shipping.
    a. Plan to ship on a Monday or Tuesday to ensure the samples DO NOT arrive on a weekend.
        i. **WARNING**: Weekend delivery can result in samples being stored in a non-refrigerated FEDEx warehouse for several days and can result in a complete loss of all samples.

2. After receiving email confirmation from the Naviaux Lab, ship the samples on at least 10 pounds of dry ice in a Styrofoam box with inner dimensions of at least 6" x 8" x 9" by FedEx overnight to:

    Dr. Robert K. Naviaux, MD, PhD
    UCSD School of Medicine
    214 Dickinson St., Room C107
    San Diego, CA 92103
    Cell: 619-993-2904

    FedEx account #1034-4986-3

Be sure to include "Room C107" in the address. Failure to do this will result in failed deliveries.