

Blood Procurement, Handling, Processing and Shipping

No changes are allowed to this document

**EARLY DETECTION RESEARCH NETWORK
GREAT LAKES NEW ENGLAND CENTER**

PROTOCOL: GLNE 007
SOP TITLE: PROCESSING BLOOD SPECIMENS
PURPOSE: To ensure technically correct, consistent, and safe methods for drawing, processing, storing, and shipping blood specimens. For additional details on Specimen Worksheets and entering specimens in VSIMS please refer to the Manual of Operations, Section 5.

Gloves must be worn at all times when handling specimens and supplies. This includes during removal of the rubber stopper from the blood tubes, centrifugation, pipetting, disposal of contaminated tubes, and cleanup of any spills. Tubes, needles, and pipets must be properly disposed of in biohazard containers, in accordance with institutional requirements.

A. Blood Kit and Associated Supplies

Contents of Blood Kit within the Specimen Collection Kit

- a) Small, biohazard plastic bag that contains:
 - i) Two 10 ml red top glass (serum) Vacutainer® tubes
 - ii) Three 10 ml brown top glass (Streck Plasma) Vacutainer tubes
- b) Two 15 ml centrifuge tube with unattached Kit ID Label
- c) Two 2d barcoded 4 ml volume serum (solid red cap) aliquot tubes to hold 2.5 ml each
- d) Twelve (12) 2d barcoded 750ul volume small serum (solid red cap) aliquot tubes to hold 300ul
- e) Two 2d barcoded 4 ml volume Streck plasma (solid blue cap) aliquot tubes to hold 2.5ml each
- f) Eighteen (18) 2d barcoded 750ul volume small Streck plasma (solid blue cap) aliquot tubes to hold 300ul
- g) 1 sided 8 1/2" by 11" Specimen Worksheets with barcode numbers - One labeled "Red Top Tubes (serum)" and one labeled "Brown Top Tubes (Streck)"

Supplies needed, but not provided in the Blood Kit

- a) Blood drawing supplies (needles, Vacutainer® adapter, tourniquet, bandages)
- b) Wet ice or ice packs
 - All Vacutainer® tubes and transfer vials get labeled by the site staff using the Kit Label provided, which corresponds to the Specimen Worksheets in the bag provided. These Vacutainer® tubes and transfer vials are supplied as a component in the combined Specimen Collection Kit.
 - The aliquot tubes are pre-assigned with 2d barcoding. Only aliquot the serum and Streck

plasma from tubes with the same Kit # that the aliquot tubes are from.

- NOTE: The aliquot tube does not contain any reference to the Kit ID. The specimen aliquot tubes cannot ever be labeled with a Kit ID. This might cause the lab to be unblinded and would be a major violation of the Study Protocol. They do have a handwritten number 1, 2 (2.5ml) and 1,2,3,...12 300ul) written on them in black sharpie.
- When processing specimens for multiple participants:
 - Pay very close attention not to mix up kit components and use separate racks.
 - It is imperative that ALL Vacutainer[®] tubes be labeled *immediately* after collection and prior to any processing.
- Never “borrow” labeled kit components from one kit to use in another.
- It is acceptable to label a Kit Bag with a participant ID label to keep things organized.
- Multiple blood draws may be performed to obtain necessary volume and/or to ensure that specimens are collected within 16 weeks before colonoscopy or prior to surgery, chemo or radiation

B. Blood Sample Collection Procedure

- a) Do not collect blood specimens unless consent has been signed.
- b) Obtain a new, unused Specimen Kit (for instructions on handling a failed specimen collection or repeat specimen collection, please refer to MOP section 5.4.1, Kit Association.)
- c) A total of 60ml of blood will be collected in the following order:
 - i) 2 red top Vacutainer[®] tubes.
 - ii) 3 brown top Vacutainer[®] tubes. Allow the tube to fill completely until blood stops flowing into the tube. Underfilling tubes with less than 5ml of blood (bottom of the label indicates 5ml fill when tube is held vertically) may lead to incorrect analytical results or poor product performance. This tube has been designed to fill with 10ml of blood. **Invert 8–10 times (180-degree turns) immediately after filling.**
- d) After collection, place the Kit ID label on each of the Vacutainer[®] tubes and ensure the worksheets match the Kit ID.
- e) On the Red Top Tubes (Serum), and Brown Top Tubes (Streck):
 - i) Label the worksheet in the appropriate racks with a Participant ID Label. This is critical for associating a Kit to a single participant.
 - ii) Enter the date and time of the draw.
 - iii) Mark the fasting status.
 - iv) Also check the appropriate racks labeled 1st/2nd Red Top Vacutainer[®] Tube, and 1st/2nd/3rd Brown Top Vacutainer[®] Tube collected to indicate if the full draw was completed.

C. Notes About The Blood Samples

- a) Wear gloves at all times when handling aliquot tubes
- b) Fill the aliquot tubes in numerical order according to the SR, or ST, numbers on the barcode labels
- c) **PLEASE NOTE THAT RED TOP VACUTAINER® TUBES ARE HANDLED AND PROCESSED DIFFERENTLY THAN BROWN TUBES**
- d) Additional processing supplies needed and supplied, but not contained in the Blood Kit
 - i) Store all kits at room temperature
 - ii) Refrigerated centrifuge with brake off feature
 - iii) Styrofoam holder for 15 ml conical tubes and blood collection tubes
 - iv) 1D Barcoded freezer racks
 - v) Site specific numbering schema for freezer racks
 - vi) If 15 ml conical tubes do not fit in your centrifuge, extra red top glass (no additive) tubes can be substituted.

D. Red Top Vacutainer Tubes:

The filled red top tubes should sit UPRIGHT IN A RACK at **room temperature for a minimum of 30 to a maximum of 60 minutes** to allow the clot to form. Process immediately or store at 4°C for up to 4 hours (On ice is acceptable for 4°C). Record appropriate times on the Specimen Worksheet.

Optimal handling until processing

- Room temperature: Minimum of 30 minutes and maximum of 60 minutes
- 4°C: Maximum of 4 hours, no minimum at 4°C required

E. Brown Vacutainer Tubes:

Once drawn the filled brown top tubes should be processed immediately or stored upright in a rack at room temperature and processed within 96 hours of collection. Record appropriate draw times on the Specimen Worksheet.

Optimal handling until processing

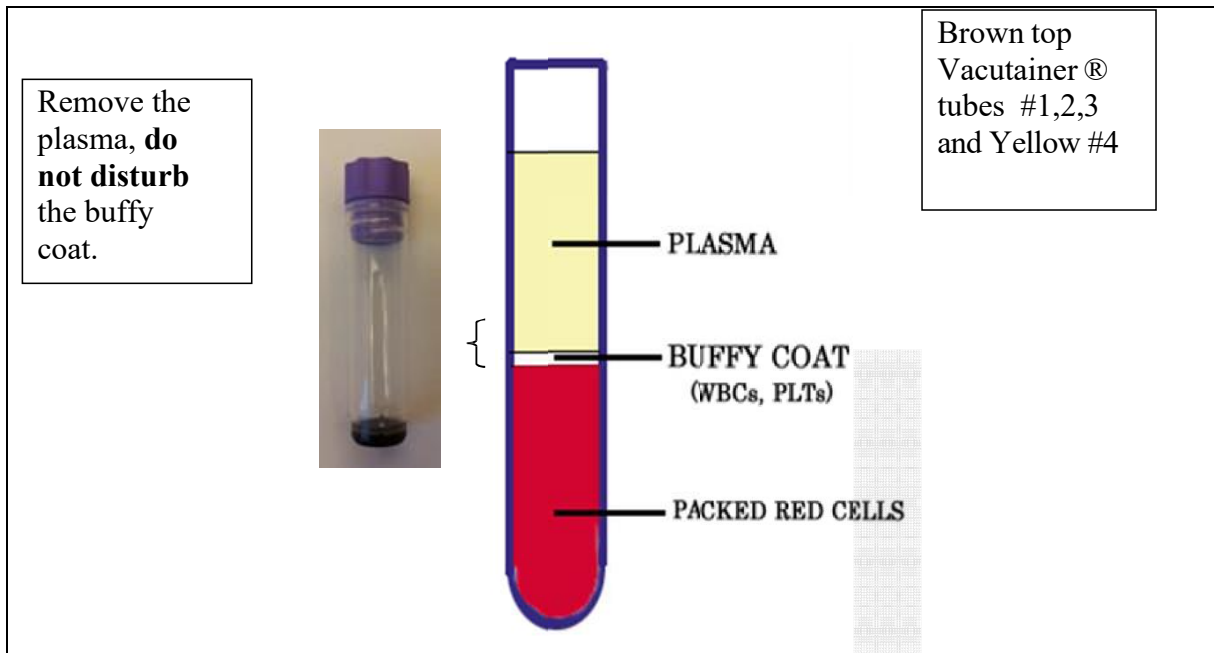
- Maximum of 96 hours at room temperature

F. Processing The Serum (Red Top) Blood Samples

- a) Keep red top Vacutainers (serum) cold at all times during processing steps. (ice or in a cold centrifuge).
- b) Minimize the time the red top tubes sit with serum separated on top of the clot as red blood cells will lyse and hemolyze the serum. Once separated, aliquot quickly.
- c) Before beginning any processing steps, assemble your supplies and allow the centrifuge to reach 4° C.
- d) Remember to store the red and tops properly based on the time of collection as described above (D)
- e) Verify that the required kit components are present, that the labels and worksheets match the tubes that you are working with.
- f) Once the centrifuge is at 4° C, place all red top Vacutainer® tubes (2) inside and make sure that they are balanced. **MAKE SURE THE BRAKE IS OFF FOR THE FIRST SPIN!**
- g) Centrifuge for 10 minutes at 4° C with the brake off at minimum force of 1300g
- h) On the Serum worksheet, record the amount of time the red Vacutainer® tubes were at room temperature and at 4° C, and the time the centrifuge was started.
- i) Uncap and place the serum , aliquot tubes in separate racks.
 - These steps should be completed before the centrifuge stops.
- j) Turn the centrifuge brake on. Re-start the centrifuge. Centrifuge the red tops for another 10 minutes at 4° C at minimum force of 1300g.
- k) When the centrifuge stops for the second time remove the red Vacutainer® tubes.
- l) Aliquot the serum into the serum aliquot tubes
 - Put 2.5 ml in the first two – 4 ml aliquot tubes
 - In the next 12 – 750ul aliquots put 300ul in each aliquot
 - Note the actual volume (if less or more than the desired volume) on the worksheet
- m) If there is serum left after this step, distribute the extra across the tubes, taking note of new volumes
- n) Cap the serum aliquot tubes tightly and place in -70°C or colder in the serum and size specific freezer racks.
- o) Completing the Serum Worksheet Note:
 - i) Update the worksheets with any new volumes (this will be changed as appropriate in the specimen entry grid in VSIMS Specimen Tracking System (LIMS), see MOP section 5.4.
 - ii) Complete Freezer time and temperature questions at the top
 - iii) Next to each barcode, check off which aliquot tubes were filled
 - iv) Mark the appropriate answers in the “Hemolyzed/lipemic/icteric?” and “SOP followed?” columns

G. Processing The Plasma (Brown Top) Blood Samples

- a) Keep the brown top Vacutainers (Streck plasma) at room temperature at all times during processing steps.
- b) Label the 15ml conical tubes with a corresponding Kit # label found in the kit bag.
- c) When ready to process, spin the 2 cell free DNA BCT Streck tubes in swinging bucket centrifuge at **1600 x g** for 10 min for cfDNA BCT Streck tubes at room temperature.
- d) Carefully transfer the supernatants (plasma) without disturbing the cellular layer from the Vacutainer tubes into one 15-mL centrifuge tube (small or large bore pipet OK).
- e) Spin the 15-mL tube in swinging bucket centrifuge at **3,220 x g**, for 10 mins for cfDNA Streck BCT tubes.
- f) It is critical that no cells are transferred, so leave ~1 mL in the bottom of the 15-mL tube to avoid any pellet that is there. If the cellular pellet is disturbed during the pipetting, the centrifugation can be repeated to re-pellet the cells
- g) Carefully transfer the supernatants (plasma) without disturbing the cellular layer from the 15-mL tube into aliquots as follows (see diagram below):
 - i) Put 2.5 ml in the first two – 4 ml aliquot tubes.
 - ii) In the next 18 – 750ul aliquots put 300ul in each aliquot
 - iii) Note the actual volume (if less or more than the desired volume) on the worksheet
- h) Cap the plasma aliquot tubes tightly and place in -70°C or colder in the plasma and size specific freezer racks.
- i) Completing Streck Plasma Worksheet
 - i) Update the worksheets with any new volumes
 - ii) Complete freezer time and temperature questions at the top
 - iii) Mark the appropriate answers in the “Hemolyzed/lipemic/icteric and SOP followed?” columns
 - iv) Record the freezer #, box# and location within box details
 - v) Next to each barcode, check off which aliquot tubes were filled
 - vi) Follow institutional procedures to dispose of contaminated supplies



H. Shipping Frozen samples:

The **frozen aliquot tubes** (serum and Streck plasma) can be shipped once a month (depending on the rate of collection) to the Central Laboratory at the University of Michigan. Multiple freezer racks can be shipped together in one larger shipping box.

Shipping Instructions for Serum and Plasma, aliquots:

PLEASE NOTE THAT TRAINING IS REQUIRED PER FEDERAL REGULATIONS TO SHIP DRY ICE. PLEASE MAKE SURE ALL PERSONNEL HAVE CERTIFICATIONS ON FILE WITH THE UM RESEARCH BASE PRIOR TO SHIPPING.

- a) "Ship" specimens in VSIMS by box number. Print all Shipping Lists generated by the system. See MOP section 5.5. Make sure that every box is full before selecting it to ship.
- b) Put the filled and locked specimen racks containing the serum and Streck plasma aliquot tube vials in a plastic Ziploc bag along with an absorbent pad/material and seal.
- c) Place bagged specimen racks (lid side up) in the bottom of the shipping box. Shipping box must be Styrofoam insert surrounded by a cardboard outer box.
- d) Fill the shipping box (around the storage racks) with as much dry ice as possible to assure the contents stay frozen even if delivery is delayed. Pelletized dry ice works best for this. Ten (10) pounds is the minimum amount of dry ice for a standard size shipment. 50 lbs total weight is the maximum accepted for shipment normally.
- e) Seal the printed Shipping Lists in a plastic bag and place on top of the dry ice before putting the insulated lid on box or on top of the insulated lid.
- f) Seal the shipping box by taping the flaps of the insulated box at the main seam across the top. **Do not tape the seams along the top edges** (required by shipping regulations to allow for the

release of carbon dioxide gas).

- g) Place a black and white label that reads “Dry Ice_kg” Complete the dry ice weight in the space “UN 1845,_____kg.” Fill in the shipped to and shipped from addresses. Place the completed dry ice label on the shipping container.
- h) Place the Human Specimens Exempt label on the shipping container
- i) Complete the provided UPS Next Day Air (red) airbill
 - i) Place completed airbill in clear pouch; place clear pouch on box.
 - ii) Do not seal the pouch (UPS will do this)
 - iii) Call 1-800-PICKUPS (1-800-742-5877) to schedule a pick up (if needed)
- j) Ship overnight (on Monday, Tuesday or Wednesday only) to the Central Lab at the University of Michigan (address above). Do not ship on Holidays.
- k) Storage at the University of Michigan. The frozen samples will be stored at –80° C.

TO REQUEST A PRE-PAID UPS LABEL FROM UM RESEARCH BASE

Option #1: Sites use their own UPS account to create shipping labels (also called waybills)

- a) Log on to www.ups.com or <https://www.campusship.ups.com> depending on the type of account you have.
- b) Select **UPS Next Day Air** as the shipping service (that is the only shipping level we will pay for).
- c) Create the label to ship to UM at:

CLASS LAB-GLNE 007
University of Michigan
1919 Green Road
Auxiliary Services Building
Ann Arbor, MI 48109
(734) 763-2461

- d) Mark “Bill Receiver” (UM) and add our account number which is: AE8404.
- e) In the Reference line 1 field enter: {XXXXXX} (the shipping label has to have this)
- f) Send a copy of the receipt to GLNE007shipping@umich.edu

Option #2: The UM staff create labels for the site. At least 24 hours prior to shipping samples to the Brenner Lab, the site coordinator will need to complete these extra steps:

- g) Complete “GLNE007 Specimen Shipping Label Request Form” (see page 3 of this bulletin).
- h) Email or fax the “GLNE007 Specimen Shipping Label Request Form” to UM as noted on the form.

- i) After receiving the request, UM staff will create shipping label(s), PDF them and email the label(s) to the email address indicated on form (please give UM at least 24 hours to complete this step).
- j) The site coordinator requesting the shipping label(s) will print the label and attach it to the package.
- k) Unless there is a regularly scheduled pick up at the site, the shipping coordinator will need to contact UPS to schedule a pickup of the package(s) from the site. Creating the label does not automatically ensure this.

Note: All other procedures for shipping specimens to UM will need to be followed (see Section 5.7 in the Manual of Operations). This is only mechanism for coordinators to receive unique and electronic labels.

GLNE007 Specimen Shipping Label Request Form – No Cover Sheet Necessary

Fax to UM research Base @ 734-764-
2566 or scan and email to
GLNE007shipping@umich.edu

Site: _____ Site Contact Person: _____

Today's Date: _____

1. Pick up address/location (please include any details that are pertinent to UPS picking up shipment):

2. Package info:

Package #	Weight (lbs)	Dry Ice (lbs)	Total Weight (lbs)
1			
2			
3			
4			
5			

3. Email address where label should be sent: _____

***NOTE:** The shipping label will be emailed to the address above. If UPS does not regularly pick up from your location, you need to call UPS directly at 1-800-PICK-UPS® to schedule a pick up. AE8404 is the account number that can be billed for any “pick up fees”.

FOR OFFICE USE ONLY

1. Labels generated by: _____

2. _____ - _____ - _____
DATE

3. Emailed to site

GLNE 007 Blood Flowchart

Serum (draw first)
2 x 10 ml red top tubes



Clot 30 min (no more than 60min) then on ice or 4 °C until centrifuged (no longer than 4 hours) Centrifuge (20 min at >1300g, 4 °C 20 min). The first 10 min with brake off, the last 10 min with brake on



2 x 4.0 ml vials with
2.5 ml serum/vial

Aliquot serum
layer into:

Red Caps

12 x 0.75 ml red vials with
300ul serum/vial

Plasma (draw second)
3 x 10 ml brown Streck tubes



Aliquot plasma layer
to a 15 ml conical tube
Centrifuge (10 min at
>1300g, 4°C brake off)



Centrifuge 15 mL
tubes for 10 min at
>1300g, 4°C with
brake on



Aliquot into:
Blue Caps



2 x 4.0 ml vials with
2.5 ml plasma/vial

18 x 0.75 ml blue vials with
300ul plasma/vial



Store in 48 sample rack
8x6

Store in 96 sample rack
12x8

Store in 48 sample rack
8x6

Store in 96 sample rack
12x8

Store all samples at -70 °C (or colder) until shipment to University of Michigan