Biospecimen Collection and Processing

May 1, 2021 Version 3.8

1. STUDY BACKGROUND AND INTRODUCTION

The Glycemia Reduction Approaches in Diabetes: A Comparative Effectiveness (GRADE) Study is a randomized clinical trial in patients with recent-onset (<10 years duration) type 2 diabetes that will compare the metabolic effects of four common anti-diabetic drugs when combined with metformin. A total of 5,000 patients within ten years of diagnosis and who are being treated with metformin at the time of recruitment will be enrolled. The subjects will be randomly assigned to one of four agents, glimepiride, sitagliptin, liraglutide and glargine, which will be added to metformin, to compare the effects among these four combinations.

The Central Biochemistry Laboratory (CBL) for the GRADE study is the Advanced Research and Diagnostic Laboratory (ARDL) at the University of Minnesota in Minneapolis. ARDL is a CLIA-certified and CAP-inspected laboratory; in addition the laboratory maintains a New York State permit allowing for testing of samples from New York State residents. The CBL will interact with the personnel at the Coordinating Center (CoC) and the Clinical Centers in collecting data to produce precise and accurate assay results. This manual primarily addresses technical issues and the quality of the laboratory specimens for the GRADE study.

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2. PREPARATION

2.1 Participant Contact

Note: Participants must be fasting for at least eight hours prior to blood collection for Baseline and Annual Visits.

Annual and Baseline visits for the GRADE Study involve the collection of a fasting sample of approximately 31 mL of blood in a total of four tubes plus a random voided urine specimen. At Baseline, there is a one-time collection of an additional 10 mL of blood for the preparation of packed cells for DNA isolation. Baseline and Annual visits at which an additional oral glucose tolerance test (OGTT) is performed involve an additional five timepoints at which 2 – 7 mL of blood is collected at each timepoint. Specimens will also be collected at the quarterly and semi-annual visits and will include: quarterly blood draws (3 mL) for hemoglobin A1c (HbA1c) measurement and semi-annual random urine collections for measurement of urinary albumin and creatinine (ACR). Additional blood may be collected for participants in sub-studies or optional study components, such as the Emotional Distress Sub-study (EDS; 2.5mL at semi-annual visits), vitamin B12 (2.5 mL at semi-annual visits) or CGM (2.5 - 5.5 mL)

2.2 Supplies Provided by the CBL

The CBL will provide an initial supply shipment that includes visit-specific kits containing collection and processing supplies and foam shipping containers used to ship specimens to the CBL. After the initial shipment of supplies is received, the field center coordinator can re-order supplies by completing the online supply re-order form. On the GRADE website, choose 'CBL', then click on 'CBL Re-ordering website.' This will link to a form where supplies can be ordered from the CBL. Alternatively, enter the following web address into your internet browser: https://sites.google.com/a/umn.edu/grade-cbl. Complete the form online and click the submit button to send the order to the CBL. Please allow 7-10 business days for receipt of supplies. Once blood collection kits are received by the field center, the field center coordinator is responsible for monitoring the expiration dates on the tubes (*blood collection tubes expire on the last day of the month printed on the label*). The supply reorder form includes an area to order replacement blood collection tubes should the tubes expire before use. Only order as many kits as you reasonably expect to use within a few months to reduce the amount of "waste" from tubes that expire.

Visit Kits

Each laboratory visit requires use of a visit-specific supply kit, which contains supplies appropriate to that visit: blood collection tubes, urine containers, cryovials with color-coded screw caps, barcoded Laboratory ID labels, plastic transfer pipettes, absorbant pads and plastic bags for shipping the specimens to the CBL. Retain the plastic bag that contains the kit supplies for use in shipping the patient's specimens to the CBL.

Barcoded Lab ID labels are included in each kit and are used to label GRADE Specimen Transmittal Forms, blood collection tubes and the processed cryovials containing serum, plasma, urine and packed cells. All of the patient's CBL assessments for a given visit will be labeled with the same Lab ID barcode. (See Appendix A.) The labels for the Baseline and Annual visits will include unique aliquot labels for the long-term storage vials. The labels will consist of a text field stating the sample type (serum, EDTA, urine, etc.) and two barcodes. The bottom barcode is the 8-digit unique Lab ID number for that participant for that visit. The top barcode is an aliquot number for the long-term storage vials. Reference the aliquotting charts in Appendix D to aliquot the correct sample type into the appropriately numbered aliquot vial. The labels to be used for blood collection tubes, the urine cup, the specimen transmittal forms, etc will have an aliquot number of "0".

The first digit of lab ID is coded to indicate GRADE visit type, as summarized in the table below.

Lab ID number format	**Visit/kit type(s)**
1xxxxxx	Baseline; DNA recollections
2xxxxxx	Final run-in; HbA1c re-tests*; final run-in re-screens
3xxxxxx	Quarterly; HbA1c confirmation
4xxxxxx	Capillary collection kit
5xxxxxx	Semi-annual; EDS/VB12 semi-annual
6xxxxxx	CGM visit ("CG" visit, CGM follow-up visit (15, 18, 21))**
7xxxxxx	Annual; CGM annual; (annual) eGFR repeat***
2200xxxxxxxxx	Microbiome****

** at direction of GRADE Study MOP section 5.4.9 – 5.4.12*

*** refer to Appendix P for details of CGM study collections.*
**** at direction of GRADE Study MOP section 10.2*
*****refer to Appendix N for details of microbiome sample collections.*

The CBL will provide supplies for *local* pregnancy testing, including a urine container, pregnancy testing device, and control material.

Specimen Transmittal Form

(See Appendix B.) A specimen transmittal form is completed at the clinical center as the specimens are collected, processed and prepared for shipment. A Lab ID label is affixed to each form. The GRADE Participant ID, the GCode, and collection date and time are also recorded on transmittal forms. A copy of this form must accompany each specimen shipment to the CBL.

Shipping Containers

The CBL will provide transport boxes for shipping specimens from the clinical sites to the CBL. After a specimen shipment is unpacked at the CBL, the large dual-temperature shipping containers will be returned to the clinical center for re-use----*do not discard the returned shipping containers!*

Bulk Supplies

The CBL will not provide bulk supplies for use in the GRADE study but will provide a small number of "Extra" tubes to have on hand at the site. If a mistake is made during processing or a blood tube does not properly fill, use a replacement tube from the "Extra" tubes supply. To ensure that your supply of kits is complete, use the online Supply Reorder Form https://sites.google.com/a/umn.edu/grade-cbl to request replacement tubes or other supplies.

3. PREGNANCY TEST

A urine pregnancy test is conducted *locally* for female patients at the Screening Visit, the Baseline Visit, and as indicated throughout the study. The pregnancy test can only be performed by personnel that work under a CLIA certification. CLIA stands for the "Clinical Laboratory Improvement Amendments" mandated by the Centers for Medicare and Medicaid Services (CMS). While the pregnancy test is considered a "CLIA-waived" test, the sites must work under at least a waiver certification. Check with your institution to determine your CLIA status and necessary compliance processes. See Appendix G for more detailed information regarding CLIA certification.

Any site personnel who will perform the urine pregnancy test must read and follow the manufacturer's instructions, without any changes to the procedure. Before any pregnancy testing can be performed, each testing personnel should read the manufacturer's instructions and take the quiz in Appendix G regarding the urine pregnancy test. Maintain the completed quiz at your site for your records.

3.1 External Quality Control

Upon receipt of pregnancy kits at the clinical site, external quality control material must be run before any of the pregnancy kits can be used. The external quality control material is provided

by the CBL and can be re-ordered using the supply reorder form website. Quality control needs to be performed only once on a new shipment of kits.

The hCG Control Set is to be used in accordance with the directions accompanying the QuickVue One-Step hCG Urine Test kits. When following these directions, the hCG Control Set is to be used in the same manner as a patient sample.

1. Gently mix the hCG Controls by inverting the vials prior to use.
2. Remove two testing devices from the packaging and place on a flat, dry surface. Label one device with “Pos” for the positive control and one device with “Neg” for the negative control.
3. Add three (3) drops (approximately 120 µL) of the Positive Control to the QuickVue One-Step hCG Urine test cassette labeled “Pos” and three (3) drops (approximately 120 µL) of the Negative Control to the QuickVue One-Step hCG Urine test cassette labeled “Neg” sample well.
4. Read test results at exactly three (3) minutes.
5. Verify *Internal Quality Control:* You should observe a blue line in the control region (C).

   POSITIVE RESULT: Two distinct lines appear, one in the control region (C), and one in the test region (T).

   NEGATIVE RESULT: One blue line appears in the control region (C). No apparent red or pink line appears in the test region (T).

   INVALID RESULT: Control line fails to appear. Repeat test with a new device.

6. Contact the CBL if the expected results are not obtained.
7. Record the results on the External QC Form (a copy can be made from Appendix G as needed throughout the study)

   a. Record the date of kit receipt in the corresponding field.
   b. Record the Lot Number of the pregnancy tests, positive control and negative control in the designated fields. The lot numbers can be found on the outer packaging of the kit/control. A column for recording the expiration date of the quality control material is also provided.
   c. Record the test result as either “POS” or “NEG” (do not use characters, such as + or -).
   d. Record the date of the external control testing.
   e. Record the initials of the person performing the testing and recording the results.
   f. Maintain the completed External QC Forms at your site.
8. External controls are stored at room temperature and are stable up until the expiration date marked on the package (date format is DD MMM YY).

3.2 Pregnancy Test Procedure

1. Obtain a random voided urine specimen.
2. Remove testing device from the test kit and place on a flat, dry surface. Label device with patient identifier.
3. Holding the dropper from the test kit vertically, transfer 3 full drops of urine to the sample well of the test device and set a timer for 3 minutes.
4. Interpret the results at exactly 3 minutes.

5. Verify *Internal Quality Control:* You should observe a blue line in the control region (C).

   POSITIVE RESULT: Two distinct lines appear, one in the control region (C), and one in the test region (T).

   NEGATIVE RESULT: One blue line appears in the control region (C). No apparent red or pink line appears in the test region (T).

   INVALID RESULT: Control line fails to appear. Repeat test with a new device.

6. Record the results on the Pregnancy Result Form (a copy can be made from Appendix G. as needed throughout the study)

   a. Record the date of testing in the "Date" field.
   b. Record the Participant's Name and Study ID Number in the designated field.
   c. Record the test result as either "POS" or "NEG" (do not use characters, such as + or -)
   d. Record the pregnancy test kit lot number. The lot number can be found on the outer packaging of the individual test cartridge.
   e. Circle either yes or no to indicate whether the blue line appeared in the control region of the test cartridge.
   f. Record the initials of the person performing the testing and recording the results.
   g. Maintain the completed Pregnancy Result Forms at your site.

4. ORGANIZATION AND SET UP

4.1 Blood Collection Tray

NOTE: Blood collection tubes are included in visit kits. The CBL does not provide the venipuncture supplies listed below.

Organize and prepare a blood collection tray. It should be made of hard, unbreakable plastic that is easy to clean and has compartments to hold the supplies listed below:

Test tube rack to hold blood collection tubes
Gloves (non-sterile)
Sterile, disposable 21-gauge butterfly needles or standard 21-gauge multisample needles
Plastic tube guides
Luer adapters for use with butterfly needles
Sterile alcohol prep pads
Gauze sponges
Tourniquets
Bandages
Crushed ice bath (cup or beaker filled with wet ice)

4.2 Organization and Set Up

Note: Patients must be fasting for at least eight hours prior to the collection of blood for fasting serum lipids, fasting glucose, fasting insulin, and fasting c-peptide measurements.

Prepare for specimen collection in the following manner, referring to the Collection and Processing Flow Diagram (Appendix D) as needed.

1. Prior to the patient's visit, remove the sheaf of barcoded Lab ID labels, the blood collection tubes and urine container (if included) from the kit appropriate to the current visit.

2. Count off one Lab ID label for each blood collection tube, cryovial, urine container (if included) and Specimen Transmittal Form. Once blood processing is complete and the transmittal form has been completed, discard any remaining Lab ID labels from the kit.

3. Affix one Lab ID label to each blood collection tube and urine container (if included). Arrange the set of blood collection tubes in a rack in *the order in which they will be collected, as follows:*

FINAL RUN-IN VISIT

a. Tube #1 2.5-mL red stopper SST tube
b. Tube #2 3-mL purple stopper EDTA tube

BASELINE

a. Tube #1 9-mL red stopper SST tube
b. Tube #2 9-mL red stopper SST tube
c. Tube #3 10-mL purple stopper EDTA tube (large)
d. Tube #4 10-mL purple stopper EDTA tube (large)
e. Tube #5 3-mL purple stopper EDTA tube **(add aprotinin)**
f. One urine container
g. **OGTT Test**
    a. 15 min – Tube #6 2-mL purple stopper EDTA tube
    b. 30 min
        i. Tube #7 4-mL purple stopper EDTA tube
        ii. Tube #8 3-mL purple stopper EDTA tube **(add aprotinin)**
    c. 60 min
        i. Tube #9 4-mL purple stopper EDTA tube
        ii. Tube #10 3-mL purple stopper EDTA tube **(add aprotinin)**
    d. 90 min – Tube #11 2-mL purple stopper EDTA tube
    e. 120 min
        i. Tube #12 4-mL purple stopper EDTA tube
        ii. Tube #13 3-mL purple stopper EDTA tube **(add aprotinin)**

ANNUAL VISITS

a. Tube #1 2.5-mL red stopper SST tube
b. Tube #2 9-mL red stopper SST tube
c. Tube #3 6-mL purple stopper EDTA tube
d. Tube #4 3-mL purple stopper EDTA tube
e. Tube #5 3-mL purple stopper EDTA tube **(add aprotinin)**
f. One urine container
g. **OGTT Test**
    a. 15 min – Tube #6 2-mL purple stopper EDTA tube

b. 30 min
    i. Tube #7 4-mL purple stopper EDTA tube
    ii. Tube #8 3-mL purple stopper EDTA tube **(add aprotinin)**
c. 60 min
    i. Tube #9 4-mL purple stopper EDTA tube
    ii. Tube #10 3-mL purple stopper EDTA tube **(add aprotinin)**
d. 90 min – Tube #11 2-mL purple stopper EDTA tube
e. 120 min
    i. Tube #12 4-mL purple stopper EDTA tube
    ii. Tube #13 3-mL purple stopper EDTA tube **(add aprotinin)**

QUARTERLY/CONFIRMATION VISITS (HbA1c only)

a. Tube #1 3-mL purple stopper EDTA tube

SEMI-ANNUAL VISIT

a. Tube #1 3-mL purple stopper EDTA tube
b. One urine container

4. Prepare a tray of the cryovials and color-coded screw caps that will contain the final samples to be shipped to the CBL. **The Lab ID labels on these vials must match those affixed to the corresponding set of blood collection tubes.**

   Affix Lab ID labels to all of the cryovials in the kit prior to blood collection.

   FINAL RUN-IN VISIT

   a. One 2-mL cryovial with red screw cap

   BASELINE VISIT

   a. Thirteen 2-mL cryovials with red screw caps labeled with aliquot numbers 1-13
   b. Ten 2-mL cryovials with purple screw caps labeled with aliquot numbers 14-23
   c. Three 2-mL cryovials with orange screw caps labeled with aliquot numbers 24-26
   d. Nine 2-mL cryovials with yellow screw caps labeled with aliquot numbers 27-35
   e. Two 5-mL blue-cap 'DNA' vials labeled with aliquot numbers 36-37
   f. OGTT Visits labeled as follows:
      i. One 2-mL cryovial with purple cap - **15 min EDTA plasma** labeled with aliquot number 38
      ii. Four 2-mL cryovials with purple caps - **30 min EDTA plasma** labeled with aliquot numbers 39-42
      iii. Three 2-mL cryovials with orange caps - **30 min EDTA + aprotinin plasma** labeled with aliquot numbers 43-45
      iv. Four 2-mL cryovials with purple caps - **60 min EDTA plasma** labeled with aliquot numbers 46-49
      v. Three 2-mL cryovials with orange caps - **60 min EDTA + aprotinin plasma** labeled with aliquot numbers 50-52
      vi. One 2-mL cryovial with purple cap - **90 min EDTA plasma** labeled with aliquot number 53
      vii. Four 2-mL cryovials with purple caps - **120 min EDTA plasma** labeled with aliquot numbers 54-57
      viii. Three 2-mL cryovials with orange caps - **120 min EDTA + aprotinin plasma** labeled with aliquot numbers 58-60

ANNUAL VISITS

a. Seven 2-mL cryovials with red screw caps labeled with aliquot numbers 1-7
b. Four 2-mL cryovials with purple screw caps labeled with aliquot numbers 8-11
c. Three 2-mL cryovials with orange screw caps labeled with aliquot numbers 12-14
d. Nine 2-mL cryovials with yellow screw caps labeled with aliquot numbers 15-23
e. OGTT Visits labeled as follows:
    i. One 2-mL cryovial with purple cap - **15 min EDTA plasma** labeled with aliquot number 24
    ii. Four 2-mL cryovials with purple caps - **30 min EDTA plasma** labeled with aliquot numbers 25-28
    iii. Three 2-mL cryovials with orange caps - **30 min EDTA + aprotinin plasma** labeled with aliquot numbers 29-31
    iv. Four 2-mL cryovials with purple caps - **60 min EDTA plasma** labeled with aliquot numbers 32-35
    v. Three 2-mL cryovials with orange caps - **60 min EDTA + aprotinin plasma** labeled with aliquot numbers 36-38
    vi. One 2-mL cryovial with purple cap - **90 min EDTA plasma** labeled with aliquot number 39
    vii. Four 2-mL cryovials with purple caps - **120 min EDTA plasma** labeled with aliquot numbers 40-43
    viii. Three 2-mL cryovials with orange caps - **120 min EDTA + aprotinin plasma** labeled with aliquot numbers 44-46

QUARTERLY VISITS (HbA1c only) – no cryovials used for quarterly visits

SEMI-ANNUAL VISITS

a. One 2-mL cryovial with yellow screw cap

5. When the patient arrives for the visit, record the Participant ID number and any other identifiers on the Specimen Transmittal Form. Also, enter the collection date and time, the participant's fasting status (if applicable) and the participant's insulin status in the appropriate spaces.

6. **IMPORTANT!** Prepare an ice bath. Fill a container approximately ¾ full with crushed ice (ice cubes can be used in place of crushed ice), and add cold water.

7. Immediately before blood collection, add 150 µL (or 0.15cc) of aprotinin (50 µL/1 mL of whole blood) to the 3-mL EDTA purple-stopper tube. Use the tuberculin syringe provided in the kit. Alternatively, aprotinin may be added to the 3mL EDTA purple-stopper tube up to 1 week (7 days) prior to use; the pre-filled tubes must be stored at refrigerated temperature (2-8^{o}C).

5. BLOOD COLLECTION

5.1 Blood Collection

Note: Patients must be fasting for at least eight hours prior to the collection of blood for fasting serum lipids, fasting glucose and fasting insulin measurements.

For detailed venipuncture instructions, see Section 5.2.

1. Perform the venipuncture filling each blood tube as completely as possible (i.e., until the vacuum is exhausted and blood flow ceases). Each visit-specific kit provides one or more blood collection tubes. Depending on the Visit, one or more of the following tubes are collected:

   Serum SST tube(s), 9-mL or 2.5-mL: After the tube is filled with blood, gently invert 8 times. Place upright in a room temperature rack. Allow blood to clot at room temperature for at least 30 minutes but not longer than 45 minutes prior to centrifugation. Set a timer.
   collected at: final run-in, baseline and annual visits

   EDTA tube, 10-mL or 6-mL (large): After the tube is filled with blood, gently invert 8 times and place upright into the crushed **ice bath** (cup or beaker containing wet ice).
   collected at: baseline and annual visits

   EDTA tube, 4-mL (for OGTT timepoint 30,60,120 min): After the tube is filled with blood, gently invert 8 times and place upright into the crushed **ice bath** (cup or beaker containing wet ice).
   collected at: baseline and annual visits

   EDTA tube, 3-mL (whole blood for A1c): After the tube is filled with blood, gently invert 8 times and place upright in a room temperature rack. **Do not centrifuge this tube!**
   collected at: final run-in, quarterly/confirmation, semi-annual and annual visits

   EDTA tube, 3-mL (for aprotinin-EDTA plasma): Immediately before blood collection, add 150 µL of aprotinin (50 µL/1 mL of whole blood) using the tuberculin syringe provided in the kit. After the tube is filled with blood, gently invert 8 times and place upright into the crushed **ice bath** (cup or beaker containing wet ice).
   collected at: baseline and annual visits

   EDTA tube, 2-mL (for OGTT timepoint 15,90 min): After the tube is filled with blood, gently invert 8 times and place upright into the crushed **ice bath** (cup or beaker containing wet ice).
   collected at: baseline and annual visits

2. Transport all tubes to the laboratory immediately for processing as directed in section 7**. Process these specimens promptly as directed so that the whole blood vial is refrigerated and the other samples frozen in a timely manner.**

5.2 Venipuncture Instructions

IMPORTANT! Consult and follow your institution's policy for venipuncture procedures.

Note: Patients must be fasting for at least eight hours prior to the collection of blood for serum lipids, fasting glucose, fasting insulin, and fasting c-peptide measurements.

DO NOT HAVE THE PATIENT MAKE A FIST IN THE HAND OF THE ARM FROM WHICH BLOOD IS TO BE DRAWN. Doing so can cause fluctuations in one or more of the analytes to measured.

Before applying the tourniquet, screw the luer adapter into the plastic tube guide. Insert the butterfly tubing onto the adapter. It is also acceptable for the venipuncture to be performed using a standard 21-gauge Vacutainer needle screwed directly into the plastic tube guide.

With jacket or sweater removed, have the patient sit upright with the sleeves rolled up to expose the antecubital fossa (inner elbow). The preferred arm to draw from is the left arm. The right arm should be used only if blood collection is not possible from the left arm. This does not mean you must stick the left arm; only do so if an adequate vein is apparent.

PRECAUTIONS WHEN USING A TOURNIQUET: The tourniquet should be on the arm for the shortest time possible. Never leave the tourniquet on for longer than one (1) minute during the actual collection. To do so may result in hemoconcentration and infiltration of blood into tissue and/or a variation in blood test values. If a tourniquet must be applied for preliminary vein selection and it remains on the arm for longer than one minute, it should be released and reapplied after a wait of two minutes. Instruct the patient not to clench his/her fist prior to the venipuncture. Doing so could cause fluctuations in the results of several of the possible analytes to be measured. If the patient has a skin problem, put the tourniquet over the patient's shirt or use a piece of gauze or paper tissue so as not to pinch the skin. Wrap the tourniquet around the arm 3 to 4 inches (7.5 to 10.0 cm) above the venipuncture site.

1. Once the vein is identified, remove alcohol prep pad from its sterile package.

2. Cleanse the vein site with the alcohol prep using a circular motion from the center to the periphery. If the tourniquet was applied during vein selection, release during the cleansing process.

3. Allow the area to dry to prevent possible hemolysis of the specimen and a burning sensation to the patient when the venipuncture is performed.

4. If venipuncture becomes difficult, the vein may need to be touched again with your gloved finger. If this happens, cleanse the site again with alcohol.

5. Re-apply the tourniquet being careful not to touch cleansed area.

Perform venipuncture:

1. Draw blood collection tubes in the following order: red-stopper SST tube(s) and then purple-stopper EDTA tube(s).

2. Grasp the patient's arm firmly. Use your thumb to draw the skin taut and anchor the vein. The thumb should be 1 or 2 inches (2.5 or 5.0 cm) below the venipuncture site.

3. With the needle bevel upward, enter the vein in a smooth continuous motion.

4. Make sure the patient's arm is in a flat or downward position while maintaining the tube below the site when the needle is in the vein. It may be helpful to have the patient make a fist with the opposite hand and place it under the elbow for support. **DO NOT HAVE THE PATIENT MAKE A FIST IN THE HAND OF THE ARM FROM WHICH BLOOD IS TO BE DRAWN**.

5. Grasp the flange of the needle holder and push the tube forward until the butt end of the needle punctures the stopper, exposing the full lumen of the needle. The tube should begin to fill with blood.

6. Remove the tourniquet as soon as blood enters the first tube. Once the draw has started, do not change the position of a tube until it is withdrawn from the needle. A tourniquet may be reapplied during collection of subsequent tubes to spare the patient a re-stick, but the tourniquet must not be on for more than 1 minute.

7. Keep a constant, slight forward pressure (in the direction of the adapter) on the end of the tube. This prevents release of the shutoff valve and stopping of blood flow.

8. Fill each blood tube as completely as possible (i.e., until the vacuum is exhausted and blood flow ceases). If a tube fills only partially, remove the tube and attach another without removing the needle from the vein.

9. When the blood flow into the collection tube ceases, remove the tube from the holder. The shutoff valve covers the point, stopping blood flow until the next tube is inserted.

10. Gently invert all blood collection tubes 8 times immediately following removal of the tube from the adapter. After inversion, place the tubes into the room temperature rack.

At the conclusion of the blood draw:

1. To remove the needle, lightly place clean gauze over venipuncture site. Remove the needle quickly and immediately apply pressure to the site with a gauze pad. Discard needle according to your institution's policy. DO NOT ATTEMPT TO RECAP NEEDLES! Have the patient hold the gauze pad firmly for one to two minutes to prevent a hematoma.

2. If blood flow stops before collecting the final tube, re-stick the patient, collecting only the unfilled tubes from the previous attempt. A tourniquet may be applied in this case but should be released if possible as soon as blood flows into the first recollected tube. As always, the tourniquet must never be on for longer than one minute.

3. If unable to obtain a complete set of blood collection tubes for the visit, call the CBL at 612-625-5040 for direction in how to proceed with recollection at a later date.

Bandaging the arm:

1. Slip the gauze pad down over the site, continuing to apply mild pressure.

2. Apply an adhesive or gauze bandage over the venipuncture site after making sure that blood flow has stopped.

Post-venipuncture:

Transport all tubes to the laboratory immediately for processing as directed in section 7**.** Process these specimens promptly as directed so that the whole blood vial is refrigerated and the other samples frozen in a timely manner.

6. URINE COLLECTION

6.1 Random Voided Urine Specimen Collection

NOTE: Urine collections should not be done if the Patient has an active UTI, febrile illness, or menses.

Minimum collection volume at BASELINE and ANNUAL visits is 20 mL urine. Minimum collection at SEMI-ANNUAL visit is 2 mL urine.

Obtain a random voided urine specimen at BASELINE, ANNUAL, and SEMI-ANNUAL visit using the container in the visit-specific kit.

7. SPECIMEN PROCESSING

7.1 Equipment Requirements

Proper processing and storage of GRADE lab specimens requires a centrifuge and a -70°C freezer. Clinical centers without access to a -70°C freezer can use a -20°C freezer. See section 7.3 for detailed instructions for the initial freeze of processed cryovials prior to storage in a -20°C freezer.

7.1.a Operating the Centrifuge

Refer to your Centrifuge Operating Manual for specific operating and balancing instructions. To achieve the recommended relative centrifugal force (RCF *minimum*: 2000 G*)* within the centrifuge, the corresponding revolutions per minute (RPM) settings will vary from one type of centrifuge to another depending on the radius of the centrifuge's rotor. Consult the centrifuge's operating manual for the appropriate RPM setting required to achieve the necessary centrifugal force (G). Centrifugation should take place at room temperature.

Clinical coordinators are encouraged to consult their own institution's laboratory for further recommendations. High-speed centrifuges with a fixed head are often programmed to spin specimens at a higher speed for a shorter period of time. Directions provided in the following sections are based on the collection tube manufacturer's recommendations when using a swing-head centrifuge.

7.2 Visit-Specific Processing

Process blood collection tubes as directed below. Refer to the collection and processing flow diagrams (Appendix D) as needed.

7.2.a FINAL RUN-IN VISIT

Stage One – Immediate Processing

1. Allow Tube #1, the 2.5-mL red stopper SST tube to clot at room temperature for at least 30 minutes, but not longer than 45 minutes prior to centrifugation. Set a timer!

2. Remove Tube #2, the 3-mL purple stopper EDTA tube from the room temperature rack and place in the refrigerator (2-8°C) until shipment to the CBL. Refrigerate the tube in an upright position, if possible.

Stage Two – Intermediate Processing (15 minutes post-collection)

1. Continue to allow Tube #1 to clot at room temperature.

Stage Three – Final Processing (30 minutes post-collection)

1. Remove Tube #1, the 2.5-mL red stopper SST tube from the room temperature rack and place into a centrifuge trunnion. Balance the centrifuge, then centrifuge at 2,000 x g for 15 minutes.

2. Post-centrifugation: Match the Lab ID label that appears on the SST tube with that on the 2-mL cryovial. Using the plastic transfer pipette included in the kit, transfer at least 0.5 mL of serum into the cryovial. Fasten a red screw cap tightly onto the cryovial.

3. Wrap the paper towel around the 2-mL cryovial, 3-mL EDTA tube. Place the wrapped tubes and the absorbent pad into the 6”x9” biohazard bag from the kit. Place this bag in the refrigerator (2-8°C).

4. **The red-cap serum vial and the 3-mL EDTA tube must be shipped to the CBL on the day of collection.** Proceed to Section 8 for packaging and shipping details.

7.2.b BASELINE VISIT

Stage One – Immediate Processing

1. Allow Tubes #1 and #2, the 9-mL red-stopper SST tubes to clot at room temperature for at least 30 minutes, but not longer than 45 minutes prior to centrifugation. Set a timer!

2. Remove Tubes #3, #4, and #5, the purple stopper EDTA tubes from the ice bath and place into a centrifuge trunnion. Balance the centrifuge trunnions, then centrifuge at 2,000 x g for 15 minutes.

3. Do not centrifuge the urine specimen. *Minimum collection volume is 20 mL.*

4. Match the Lab ID label on the urine container with those on the nine 2-mL cryovials labeled with aliquots #27 – 35. Using the plastic transfer pipette included in the kit, transfer approximately 2 mL of urine into each vial.

5. Tightly fasten yellow screw caps onto the cryovials and place in a rack in the refrigerator (2-8°C) until transfer to -70°C freezer. Re-cap the urine container and discard.

Stage Two – Intermediate Processing (15 minutes post-collection)

1. Continue to allow Tubes #1 and #2 to clot at room temperature.

2. Following centrifugation of Tubes #3, #4, and #5:
   a. **Tubes #3 and #4 (Consult Appendix D for detailed diagram and instructions)**:
      i. Post-centrifugation: Match the Lab ID labels that appear on the tubes with those on the 2-mL cryovials labeled with aliquots #14 – 23 and 5-mL DNA transport vials labeled with aliquots #36 – 37.
      ii. Taking care not to disturb the cell layer, remove the clear plasma supernatant and transfer 0.5 mL plasma into each of 10 labeled 2-mL cryovials (#14 – 23). Aspirate slowly starting at the top of the plasma. Leave a ½ inch layer of plasma above the buffy coat-red blood cell layers. It is important to withdraw only the plasma and none of the buffy coat (containing white blood cells and platelets) that forms at the cell-plasma interface following centrifugation. If some of the buffy coat is accidentally aspirated while removing the plasma, return the buffy coat and plasma to the original tube and re-centrifuge the tube under the initial processing conditions. Fasten **purple** screw caps tightly onto the cryovials.
      iii. Using the same plastic pipette, slowly aspirate the remaining ½ inch layer of plasma, the buffy coat, and some of the remaining red cells from the tube. Take care not to aspirate the buffy coat into the bulb of the pipette. "Ring" the tube with the pipette by carefully aspirating along the wall at the buffy coat layer to ensure maximum transfer. Dispense into the 5-mL DNA transport vial (#36).
      iv. Still using the same pipette, go back and transfer all of the remaining packed red cells from the tube to the same 5-mL DNA vial. This step will ensure that the entire buffy coat is adequately rinsed from the pipette.
      v. Repeat with the second EDTA tube (tube #4) into aliquot #37. Fasten **blue** caps onto the 5-mL DNA transport vials.
      vi. Re-stopper the empty blood collection tubes and discard.

   b. **Tube #5 (EDTA + aprotinin tube)**
      i. Post-centrifugation: Match the Lab ID labels that appear on the tube with those on the 2-mL cryovials labeled with aliquots #24 – 26.
      ii. Using the plastic transfer pipette included in the kit, transfer 0.5 mL of plasma into three labeled 2-mL cryovials (#24-26). Re-stopper the empty blood collection tube and discard.
      iii. Tightly fasten **orange** screw caps onto the transport vials.

Stage Three – Final Processing (30 minutes post-collection)

1. Remove Tubes #1 and #2 from the room temperature rack and place into a centrifuge trunnion. Balance the centrifuge, then centrifuge at 2,000 x g for 15 minutes.

2. Post-centrifugation of SST tubes: Match the Lab ID label that appears on the SST tube with that on the 2-mL cryovials labeled with aliquots #1 – 13. Using the plastic transfer pipette included in the kit, transfer 1.0 mL of serum into the first cryovial (#1). Then, transfer 0.5 mL of serum into the 12 remaining 2-mL cryovials (#2 – 13).

Tightly fasten red screw caps on all of the cryovials. Re-stopper the empty blood collection tubes and discard.

3. Retrieve the urine cryovials from the refrigerator. Place the aliquot rack containing *all* of the participant's cryovials into a -70°C freezer (or see section 7.3 for clinical centers without access to a -70°C freezer). The aliquots should freeze upright so the specimen does not freeze in the cap.

4. Once frozen (approximately 60 minutes), the vials can be transferred to the 6" x 9" biohazard bags from the kit. Separate serum aliquot 1, plasma aliquot 14 and urine aliquot 27 from all other vials. Place the remainder of all of the red-capped cryovials in one 6" x 9" biohazard bag. Place the remainder of all of the purple-capped and orange-capped cryovials together in one 6" x 9" biohazard bag. Place the remainder of all of the yellow-capped urine cryovials in one 6" x 9" biohazard bag. Place both of the blue-capped DNA packed cell vials AND serum aliquot 1, plasma aliquot 14 and urine aliquot 27 in one 6" x 9" biohazard bag.

5. Place one absorbent pad into each biohazard bag. This serves to soak up any leaks and spills that may occur during shipping.

6. Place all of the 6" x 9" biohazard bags (including the 6" x 9" biohazard bag containing the OGTT specimens) into the large 9" x 12" plastic ziplock bag included in the kit, so that all of the participant's specimens are in one large bag.

5. Place the bag containing the cryovials in a -70°C freezer (or -20°C freezer if no access to -70°C freezer) until shipment to the CBL. Proceed to Section 8 for packaging and shipping details.

7.2.c ANNUAL VISITS

Stage One – Immediate Processing

1. Allow Tubes #1 and #2, the **SST tubes** to clot at room temperature for at least 30 minutes, but not longer than 45 minutes prior to centrifugation. Set a timer!

2. Remove Tubes #3 and #5, the 6-mL purple stopper **EDTA tube (large)** and the 3-mL **EDTA tube with aprotinin** from the ice bath and place into a centrifuge trunnion. Balance the centrifuge trunnions, then centrifuge at 2,000 x g for 15 minutes.

3. Remove Tube #4, the 3-mL purple stopper **EDTA tube (small)** from the room temperature rack and place in the refrigerator (2-8°C) until shipment to the CBL. Refrigerate the tube in an upright position, if possible.

4. Do not centrifuge the urine specimen. *Minimum collection volume is 20 mL.*

5. Match the Lab ID label on the urine container with those on the nine 2-mL cryovials labeled with aliquots #15 – 23. Using the plastic transfer pipette included in the kit, transfer approximately 2 mL of urine into each cryovial.

6. Tightly fasten yellow screw caps onto the cryovials and place in a rack in the refrigerator (2-8°C) until transfer to -70°C freezer. Re-cap the urine container and discard.

Stage Two – Intermediate Processing (15 minutes post-collection)

1. Continue to allow Tubes #1 and #2 to clot at room temperature.

2. Following centrifugation of Tubes #3 and #5:
   a. **Tube #3**:
      i. Post-centrifugation: Match the Lab ID labels that appear on the tubes with those on the 2-mL cryovials labeled with aliquots #8 – 11.
      ii. Using the plastic transfer pipette included in the kit, transfer 0.5 mL of plasma into four labeled 2-mL cryovials (#8 -11). Re-stopper the empty blood collection tube and discard.
      iii. Tightly fasten **purple** screw caps onto the cryovials.
      iv. Re-stopper the empty blood collection tube and discard.

   b. **Tube #5 (EDTA + aprotinin tube)**
      i. Post-centrifugation: Match the Lab ID labels that appear on the tube with those on the 2-mL cryovials labeled with aliquots #12 – 14.
      ii. Using the plastic transfer pipette included in the kit, transfer 0.5 mL of plasma into three labeled 2-mL cryovials (#12 – 14). Re-stopper the empty blood collection tube and discard.
      iii. Tightly fasten **orange** screw caps onto the cryovials.

Stage Three – Final Processing (30 minutes post-collection)

1. Remove Tubes #1 and #2 from the room temperature rack and place into a centrifuge trunnion. Balance the centrifuge, then centrifuge at 2,000 x g for 15 minutes.

2. Post-centrifugation of SST tubes: Match the Lab ID label that appears on the SST tube with that on the 2-mL cryovials labeled with aliquots #1 – 7. Using the plastic transfer pipette included in the kit, transfer 1.0 mL of serum into the first cryovial (#1). Then, transfer 0.5 mL of serum into the six remaining 2-mL cryovials (#2 – 7). Re-stopper the empty blood collection tubes and discard.

3. Tightly fasten red screw caps on the cryovials.

4. Retrieve the urine cryovials from the refrigerator. Place the aliquot rack containing *all* of the participant's cryovials into a -70°C freezer (or see section 7.3 for clinical centers without access to a -70°C freezer). The aliquots should freeze upright so that the specimen does not freeze in the cap.

5. Once frozen (approximately 60 minutes), the vials can be transferred to the 6" x 9" biohazard bags from the kit. For the fasting sample vials, separate serum aliquot 1, plasma aliquot 8 and urine aliquot 15 from all other vials and place in one 6" x 9" biohazard bag. For annual visits that include OGTT collection: place the remainder of all of the red-capped cryovials in one 6" x 9" biohazard bag; place the remainder of all of the purple-capped and orange-capped cryovials together in one 6" x 9"

biohazard bag; place the remainder of all of the yellow-capped urine cryovials in one 6” x 9” biohazard bag. For annual visits that do NOT include OGTT collection: place the remainder of all red-, purple-, orange- and yellow-capped vials into one 6” x 9” biohazard bag (all will be in the same bag). See section 7.2.d for packaging of OGTT vials.

6. Place one absorbent pad into each biohazard bag. This serves to soak up any leaks and spills that may occur during shipping.

7. Place all of the 6” x 9” biohazard bags (including the 6” x 9” biohazard bag containing the OGTT specimens) into the large 9” x 12” plastic ziplock bag included in the kit, so that all of the participant’s specimens are in one large bag.

8. Place the bag containing the cryovials in a -70°C freezer (or -20°C freezer if no access to -70°C freezer) until shipment to the CBL. Proceed to Section 8 for packaging and shipping details.

7.2.d OGTT VISITS

Reminder: Centrifuge collection tubes from each timepoint immediately after collection. Do not wait until the end of the 2-hour OGTT to centrifuge all 8 tubes from all timepoints together.

Stage One – Immediate Processing

1. **The 2-mL cryovials are labeled according to specimen type (EDTA plasma or EDTA + aprotinin plasma) and OGTT timepoint (15 min, 30 min, 60 min, 90 min, and 120 min).**

2. Remove the blood collection tubes from the ice bath. Place the tubes in a centrifuge trunnion. Balance the centrifuge, then centrifuge at 2,000 x g for 15 minutes.

Stage Two – Final Processing (15 minutes post-collection)

1. Post-centrifugation of the EDTA: Match the Lab ID label on the tube to the 2-mL cryovials. **Also, match the correct specimen type (EDTA plasma or EDTA + aprotinin plasma) and the OGTT timepoint.**

   OGTT Timepoints 15 min and 90 min

   a. Using the plastic transfer pipette included in the kit, transfer 0.5 mL of plasma into the 2-mL cryovial. Re-stopper the empty blood collection tube and discard.
   b. Tightly fasten a purple screw cap onto the cryovial.

   OGTT Timepoints 30 min, 60 min, and 120 min

   c. Using the plastic transfer pipette included in the kit, transfer 0.5 mL of the **EDTA only** plasma into the four labeled 2-mL cryovials. Re-stopper the empty blood collection tube and discard. Tightly fasten the cryovials with purple screw caps.

d. Using the plastic transfer pipette included in the kit, transfer 0.5 mL of the **EDTA + aprotinin** plasma into the three labeled 2-mL cryovials. Re-stopper the empty blood collection tube and discard. Tightly fasten the cryovials with orange screw caps.

2. Place the aliquot rack containing the cryovials into a -70°C freezer (or see section 7.3 for clinical centers without access to a -70°C freezer). The aliquots should freeze upright so the specimen does not freeze in the cap. Once frozen (approximately 60 minutes), the vials can be transferred to the 6" x 9" plastic bag from the kit.

3. At the conclusion of the OGTT test, place all of the frozen specimen cryovials for one participant into a 6" x 9" biohazard bag containing an absorbent pad. Place this bag in the large 9" x 12" plastic ziplock bag containing the rest of the same participant's specimens, so that all of the participant's specimens are in one large bag.

4. Place the bag containing the cryovials in a -70°C freezer (or -20°C freezer if no access to -70°C freezer) until shipment to the CBL. Proceed to Section 8 for packaging and shipping details.

7.2.e QUARTERLY/CONFIRMATION VISITS

Remove Tube #1, the 3-mL purple stopper **EDTA tube (small)** from the room temperature rack and place in the refrigerator (2-8°C) until shipment to the CBL. Refrigerate the tube in an upright position, if possible. Proceed to Section 8 for packaging and shipping details.

7.2.f SEMI-ANNUAL VISITS

1. Remove Tube #1, the 3-mL purple stopper **EDTA tube (small)** from the room temperature rack and place in the refrigerator (2-8°C) until shipment to the CBL. Refrigerate the tube in an upright position, if possible.

2. Do not centrifuge the urine specimen. *Minimum collection volume is 2 mL.*

3. Match the Lab ID label on the urine container with that on the 2-mL cryovial. Using the plastic transfer pipette included in the kit, transfer approximately 2 mL of urine into the cryovial. Tightly fasten a yellow screw cap onto the cryovial. Re-cap the urine container and discard.

4. Place the aliquot rack containing the urine cryovial into a -70°C freezer (or see section 7.3 for clinical centers without access to a -70°C freezer). The aliquot should freeze upright so the specimen does not freeze in the cap. Once frozen (approximately 60 minutes), transfer the cryovial to the 6" x 9" plastic bag from the kit.

5. Place one absorbent pad into the biohazard bag. This serves to soak up any leaks and spills that may occur during shipping.

6. Place the bag containing the cryovial in a -70°C freezer (or -20°C freezer if no access to -70°C freezer) until shipment to the CBL. Proceed to Section 8 for packaging and shipping details.

7.3 FREEZER STORAGE

IMPORTANT! Processed transport vials containing serum, plasma, and urine must be placed into the freezer within 90 minutes of blood collection. Samples must be placed in the -70°C freezer for a minimum of 60 minutes prior to packaging for shipment to the CBL. They must be thoroughly frozen prior to shipment. Do not allow the specimens to thaw at any time.

For clinical centers without access to -70°C freezer: Place some dry ice (preferably in pellet form) into the open foam box from a shipping assembly. Place the processed cryovial rack into a plastic bag. Expel the air from the bag and place it on the dry ice in an upright position. Add more dry ice to cover the portion of the bag that contains the vials. Keeping the rack upright, place the open box containing the vials and dry ice into the -20°C freezer for a minimum of 60 minutes. Once frozen for at least 60 minutes, transfer vials to 6" x 9" biohazard bag(s) as described in the Final Processing instructions for each visit. Immediately package for shipment.

8. STORAGE, PACKAGING, AND SHIPPING OF SPECIMENS

8.1 Specimen Storage Prior to Shipping

FINAL RUN-IN VISIT

Samples from the Final Run-in Visit must be shipped to the CBL on the day of collection.

Any deviations from same-day shipping should be discussed with the CBL. Store the EDTA whole blood tube and the serum cryovial refrigerated at 2-8°C until packaged for shipment to the CBL via overnight courier.

ALL OTHER VISITS

Samples from a Confirmation Visit should be shipped to the CBL on the day of collection. If sites are unable to ship on the day of collection, whole blood samples can be shipped within 10 days of collection.

It is preferred that lab specimens from all other visits are shipped to the CBL on the day of collection. However, if sites are unable to ship the day of collection, samples must be shipped within 10 days of collection. Do not ship a participant's refrigerated samples separate from the frozen samples. Always ship the entire sample set for a participant together in a dual-temperature shipper (see section 8.5 for details).

Store the EDTA whole blood tube refrigerated at 2-8°C until packaged for shipment to the CBL via overnight courier.

Store the cryovials containing serum, plasma, urine and/or packed cells frozen at -70°C (or -20°C freezer if no access to -70°C freezer) prior to shipping to the CBL via overnight courier. Ship each participant's frozen specimens as a set enclosed in a single large plastic bag--one

bag per participant--as directed in the previous section. No more than 2 participants' specimen sets should be shipped in the same foam shipping container.

Shipments may be sent **Monday through Friday,** being mindful of weeks in which a holiday occurs. Any shipment deviations or questions should be discussed directly with the CBL.

8.2 Reviewing the GRADE Specimen Transmittal Forms

1. Check the Specimen Transmittal Form against the processed cryovials as they are packaged for shipment. Ensure that the Participant ID and barcode and other identifiers have been recorded on the form and that the correct Lab ID label has been affixed to the form. Also, ensure that the visit number and collection date and time(s) are recorded on the form.

2. Confirm that the number of filled cryovials contained in the plastic bag corresponds to the number of cryovials noted on the Specimen Transmittal Form as 'shipped.' If shipping an incomplete sample set of cryovials, record the number of missing cryovials and their cap colors with a description of the problem(s) in the Comments section of the STF. If shipping empty/unfilled cryovials from the collection to the CBL, these are not counted as 'shipped' vials; they should be counted as part of the 'missing' cryovials. Do not affix the cap to any empty/unfilled cryovial; these vials should be shipped without their caps.

3. Retain photocopies of the GRADE Specimen Transmittal Form at your clinical center.

8.3 Packaging Refrigerated Specimens

NOTE: Remove any patient identifiers other than GRADE labels from all shipping forms, tubes, vials, containers and plastic bags. The CBL must remain blinded to patients' identifying information for HIPAA and IRB purposes.

Ship the refrigerated specimens via overnight courier to the CBL. Shipments may be sent **Monday through Friday.** Any shipment deviations or questions should be discussed directly with the CBL. Prepare for specimen shipment in the following manner, referring to the shipping flow diagram (Appendix E) as needed.

NOTE: One or more refrigerant packs should be stored frozen, ready for shipment, preferably inside the open foam box (smaller shipper) to avoid later difficulty in fitting the rigid pack into the foam box. Place refrigerant pack into the freezer at least one day prior to shipping --- it must be frozen solid at the time of shipment.

NOTE: It is acceptable to package more than one participant's samples together for shipping to the CBL. However, please follow these general rules: 1) only 1 participant's refrigerated temperature samples should be packed per small foam box; 2) each participant's samples must be in their own plastic bags, as described in Section 7; 3) up to 3 participants' refrigerated temperature shipping containers may be shipped in 1 *FedEX UN3373 Clinical Pak*; 4) do not package refrigerated temperature-only visits (Final run-in and Quarterly/Confirmation visits) with frozen sample-containing visits (Baseline, Semi-annual and Annual); 5) any combination of refrigerated temperature-only visits may be shipped in the same *FedEX UN3373 Clinical Pak*;

and 6) any combination of frozen sample-containing visits may be shipped in the same dual shipping container.

1. FINAL RUN-IN VISIT ONLY: Wrap the paper towel from the kit securely around the EDTA purple-stopper tube and the 2-mL serum cryovial to insulate them from direct contact with the frozen refrigerant pack.

   QUARTERLY/CONFIRMATION and ANNUAL VISITS: Wrap paper towel from the kit securely around the whole blood EDTA purple-stopper tube to insulate it from direct contact with the frozen refrigerant pack.

2. Place the wrapped packet into the 6" x 9" biohazard plastic storage bag. Place one of the absorbent pads from the kit into the bag and seal the bag tightly. The absorbent pad serves to soak up any leakage in transit --- it does not provide any insulation for the vial.

3. Place a frozen refrigerant pack on the bottom of the foam box.

4. Place the biohazard bag containing the wrapped tube on top of the frozen refrigerant pack. Only 1 participant's samples should be packaged into 1 foam box. Place the lid on the foam box and insert it into its cardboard sleeve (carton) and close the lid or tab.

5. Place the completed Specimen Transmittal Form in the 9" x 12" plastic ziplock bag.

6. FINAL RUN-IN and QUARTERLY/CONFIRMATION VISITS: Place the shipper along with the completed Specimen Transmittal Form into a *FedEX UN3373 Clinical Pak*, then close and seal the Pak. Up to 3 participants' shippers may be shipped in 1 Clinical Pak. Proceed to section 9.0 Shipping Instructions.

   SEMI-ANNUAL and ANNUAL VISITS: Proceed to section 8.5 Final Packaging Instructions – Dual Shipping Container

8.4 Packaging Frozen Specimens

BASELINE, SEMI-ANNUAL and ANNUAL VISITS

NOTE: Remove any patient identifiers other than GRADE labels from all shipping forms, tubes, vials, containers and plastic bags. The CBL must remain blinded to patients' identifying information for HIPAA and IRB purposes.

Prepare the specimen shipment in the following manner, referring to the shipping flow diagram (Appendix E) as needed. Ship the frozen samples to the CBL via overnight courier. Shipments may be sent **Monday through Friday.** Any shipment deviations or questions should be discussed directly with the CBL.

1. Retrieve the large plastic storage bag containing the participant's frozen specimen set from the -70°C freezer. Double-check at this time to be sure that all the cryovials within each participant's bag share the same Lab ID number.

2. Place a layer of dry ice inside the large foam box. Place one plastic bag containing a participant's frozen sample set on top of the dry ice.

3. Layer more dry ice on top of the bag. If more than one participant's sample set is to be shipped in the same box, layer dry ice in between each plastic bag. Up to 2 participants' frozen samples may be shipped in 1 foam box. A total of approximately 5 lbs dry ice should be used per foam box.

4. Place the lid on the foam box. To secure the lid to the box, apply a single strip of strapping tape over the top of the container --- the strip should extend approximately 6 inches down each side as shown in Appendix E. Do not encircle the box with strapping tape. Do not apply strapping tape along the seam where the lid meets the box. Insert the foam box down into its cardboard sleeve (carton).

5. Place the Specimen Transmittal Forms into the 9" x 12" plastic ziplock bag and place this on top of the large foam box.

8.5 Final Packaging Instructions – Dual Shipping Container

SEMI-ANNUAL and ANNUAL VISITS

Place the small shipper containing the refrigerated whole blood specimen on top of the large foam box. If shipping 2 participants' frozen samples in the same shipment, the refrigerated small shippers for each must also be included on top of the large foam box.

BASELINE, SEMI-ANNUAL and ANNUAL VISITS

Close and seal the outer cardboard carton tightly with strapping tape as shown in Appendix E. Proceed to section 9 for shipping instructions.

9. SHIPPING INSTRUCTIONS

9.1 Shipping Labels

Shipping containers are sent to the CBL by FedEx 'Priority Overnight' service to ensure receipt within 24 hours. After a specimen shipment is unpacked at the CBL, the dual-temperature shipping containers will be returned to the field center for reuse --- do not discard the returned containers.

Federal law requires that you know and comply with the specific packaging requirements for shipping 'Hazardous Materials'. You are responsible for obtaining and maintaining the appropriate training to meet the federal regulatory requirements.

IMPORTANT! Prior to sending the first specimen shipment, consult your institution's shipping department regarding any further labeling or packaging requirements.

1. IATA Regulations require that a label be affixed to the outside of shipping containers that reads: **'UN3373 – Biological Substance Category B'**.

a. **Refrigerated specimen shipments only:** If a *FedEx UN3373 Clinical Pak* is not used, you must affix a **'UN3373 – Biological Substance Category B'** sticker to the outside of the shipping container.

b. **Dual specimen shipments (containing both refrigerated and frozen specimens):** You must affix both a **'UN3373 – Biological Substance Category B'** sticker and a completed **dry ice label** to the outside of the box. Affix the UN3373 label so that the lines surrounding 'UN3373' are placed at a 45^{o} angle to the edges of the shipper. Record the Shipper's name and address and the Consignee's name and address and the total weight of the shipment in kg on the dry ice label. Do not write within the dotted boundaries of the diamond shape.

2. Check your shipping boxes to ensure they are properly labeled in order to avoid any interruption in shipment. IMPORTANT! Consult your institution's Shipping Department for any further labeling or packaging requirements.

3. Send shipping containers to the CBL via **'Priority Overnight'** FedEx Courier Service to ensure receipt within 24 hours. **Ship Monday through Friday only.** Any shipment deviations or questions should be discussed directly with the CBL.

4. Contact Federal Express (1-800-GO-FEDEX) for pickup or use the designated Federal Express pickup system at your clinical center.

9.2 Completing the FedEx airbill

The CBL will provide pre-printed, barcoded airbills for shipping refrigerated temperature specimens and for shipping frozen specimens (dry ice).

If not already pre-printed on the airbill, please write on the airbill: "UN3373 Biological Substance Category B."

Affix the self-stick airbill firmly to the top of the shipping container, ensuring that the correct version of the airbill is applied to the correct shipping container. Affixing a frozen temp/dry ice airbill to a refrigerated temperature shipment or a refrigerated temp airbill to a frozen temp/dry ice shipment could result in FedEx rejecting the shipment or in substantial fines.

See Appendix Q for example of the airbills to use for refrigerated and frozen temperature shipments.

If you need to complete a blank paper airbill, please contact the CBL directly for instructions.

IMPORTANT: Please double-check your shipping boxes to ensure that they are properly labeled in order to avoid any shipment delays or fines.

10. ANALYSIS AND RESULTS REPORTING

Specimens will be analyzed upon receipt at the CBL. Results will be transmitted electronically to the GRADE Study Coordinating Center (CoC). Hard copy results reports will be auto-faxed to

the site coordinator at the clinical center (see Appendix F). See Appendix H for a list of all lab tests and reference ranges performed by the GRADE CBL.

Contact the CBL at 612-625-5040 if you do not receive a results report within 3 to 5 days of specimen shipment. Please notify the CBL Laboratory Study Coordinator via email of changes to your clinical center's fax number, fax addressee's name and/or the name of your clinical center.

11. REMOTE SAMPLE COLLECTIONS

11.1 HbA1c Capillary Collection Kits—Collections Performed in US

Collection kits are available to mail to a participant's home for the self-collection of blood for HbA1c analysis. A small volume of blood is obtained from a fingerstick, then transferred to a special collection tube containing a preservative solution; the packaged sample is sent by the participant to the CBL for analysis. Because the mailers for these collections include pre-paid postage, the instructions apply only to collections that are mailed within the US, including Hawaii and Alaska. For collections done in another country or from a US territory, see section 11.2.

1. Request the HbA1c capillary collection kit from the CBL

   a. The blue-top collection tube has an expiration date. The expiration date is printed on a label affixed to the outside bag containing each kit.

2. Prepare kit for participant

   a. Remove blue-top collection tube from kit and affix the 'vial' lab ID label (4xxxxxxx series) from label set to tube.

      i. Print CAPLSTF, complete the participant identification and visit information, then affix a lab ID label from the label set. Make a photocopy of the CAPLSTF to retain at the clinical center and send the original with the kit to the participant.

      ii. If capillary kit is sent to participant for an at-home collection for confirmation of an A1c > 9.0%, complete CAPLSTF. Enter "IN" as the visit code on the CAPLSTF prior to sending kit and STF to participant.

   b. Discard remainder of lab ID labels at site; do not send to participant.

3. Mail kit to the participant

   a. Mail the collection kit, including the pre-labeled blue-top collection tube, partially completed STF, participant instruction sheet and cover letter to the participant.

   b. A box or padded mailer is recommended to send the kit, although the CBL does not provide the supplies for sending the kit from the clinical site to the participant. Postage for sending the kit to the participant is also the responsibility of the clinical site; the CBL does not provide this.

11.2 HbA1c Capillary Collection Kits—Collections Performed Outside the US

When a participant resides outside the 50 US states, a modified collection kit for the self-collection of blood for HbA1c analysis is available. Rather than shipping through the US Postal Service (USPS), the sample collections will be shipped to the CBL via FedEx. FedEx will be used because of the varying postage costs for international mail and to expedite shipment of these samples to the CBL. Before requesting this kit, please contact the Coordinating Center and the CBL to discuss the collection; FedEx does not operate in all countries.

1. Request the HbA1c capillary collection kit from the CBL

    a. Clearly indicate that the requested kit is for an international capillary collection.

    b. The blue-top collection tube has an expiration date. The expiration date is printed on a label affixed to the outside bag containing each kit.

    c. The kit will contain all items included with a usual capillary collection kit, with the exception of the postage-paid mailer; a set of modified participant instructions will also be included that provide details about the packaging and shipping of the sample. The kit will include the following shipping supplies in place of the postage-paid mailer:

        i. 11” x 9” x 10” Styrofoam container with cardboard box
        ii. 1- 8oz. gel pack
        iii. Paper towel
        iv. FedEx international airbill
        v. FedEx international airbill and documentation pouch
        vi. UN3373 Biological substance Category B label
        vii. Commercial invoice (3 copies)
        viii. *The participant will need to supply some newspaper to fill remaining empty space in the shipper*

2. Prepare kit for participant

    a. Remove blue-top collection tube from kit and affix the ‘vial’ lab ID label from label set to tube.

    b. Print CAPLSTF, complete as much of the information as possible, then affix a lab ID label from the label set. Make a photocopy of the STF to retain at the clinical center and send the original with the kit to the participant.

        i. Note: If capillary kit is sent to participant for an at-home collection for confirmation of an A1c > 9.0%, complete CAPLSTF. Enter “IN” as the visit code on the CAPLSTF prior to sending kit and STF to participant.

    c. Discard remainder of lab ID labels at site; do not send to participant.

    d. Complete sections on the commercial invoice (all copies, as needed) as follows:

i. EXPORTER: [This will be the participant's/shipper's information.] Complete Contact Name, Telephone No., Email, Address and Country information. No other boxes in this section need to be completed. The participant could also complete this information.
ii. CONSIGNEE: [This will be the clinic's information.] Complete Contact Name, Telephone No., Email, Address and Country information. No other boxes in this section need to be completed.
iii. SHIP DATE: The participant will complete this information.
iv. ORIGINATOR OR NAME OF COMPANY REPRESENTATIVE…: Participant's/shipper's printed name.
v. SIGNATURE/TITLE/DATE: The participant/shipper will complete this information.

e. Complete International FedEx airbill with:

i. Field #1 ('From'): Leave blank for participant to complete when shipping.
ii. Field #2 ('To'): Print the <u>your clinic's address</u> and phone number in this field.
iii. Field #3 (Shipment information): Total packages = 1. Total weight = **<participant to complete when shipping>**. DIM = 11 x 9 x 10 inches. Description: 1 human blood sample not known to be infectious or contagious; to be used for medical research. Value for customs = 12.50. Total declared value for carriage = 12.50. Total value for customs = $12.50 USD. Do not record anything for harmonized code or country of manufacture, or any of the information in the EEI/SED section at the bottom left.
iv. Field #4 (Express Package Service): Check 'FedEx IntlPriority' only--do not check any other option.
v. Field #5 (Packaging): Check 'Other' and enter 'box' in blank space
vi. Field #6 (Special Handling): Do not check any boxes.
vii. Field #7a (Payment - Bill Transportation to:): Check the 'Third party' box. Record the FedEx account number.
viii. Field #7b (Payment - Bill duties and taxes to:): Check the 'Third party' box. Record the FedEx account number.
ix. Field #8 (Your Internal Billing Reference Information): Print 'GRADE'
x. Field #9 (Signature): Leave blank for participant to complete when shipping.

f. Repackage kit with labeled blue-top vial, partially-completed STF, partially-completed commercial invoice and partially-completed International FedEx airbill into the 11" x 9" x 10" Styrofoam container with cardboard box. Give all this to participant, either in person or by shipping box to participant.

3. The participant will mail the collected sample <u>to your clinic</u>. This is because the participant will need to record their name and address information in various sections of the airbill and commercial invoice. The CBL is not allowed to know any identifying information about the samples it receives.

a. Upon receipt of the sample at the clinic, sample condition should be noted (i.e. was the sample packaged appropriately? was the gel pack still cold/frozen?). Record any of these comments on the CAPLSTF and date/initial the comment.

b. Re-package the blue-top vial and CAPLSTF into a small Styrofoam shipper containing a frozen gel pack, place in the FedEx clinical pak and send to the CBL.

12. USING CAPILLARY COLLECTION KITS IN-CLINIC

If a whole blood HbA1c sample cannot be collected in-clinic at a regularly scheduled study visit due to venous access issues, a capillary collection may be completed in place of the (venous) whole blood collection. However, before using the kit, at least one attempt to obtain a venous sample must be made, and in an experienced coordinator/phlebotomists' judgment, it must be judged highly unlikely that a venous sample could be obtained successfully. **Please note you must always first try to obtain the sample using the regular venous method.**

12.1 In-clinic procedures

1. Label blue-top vial with lab ID from the capillary kit (4xxxxxxx series label). Use participant instructions to guide your collection of the capillary blood sample.

2. Document collection using the CAPLSTF. Record participant information and date/time of collection. Affix label from the capillary kit (4xxxxxxx series label) to STF.

3. If a urine sample is collected at the visit, the appropriate visit STF must also be completed and sent with the sample to document the urine collection.

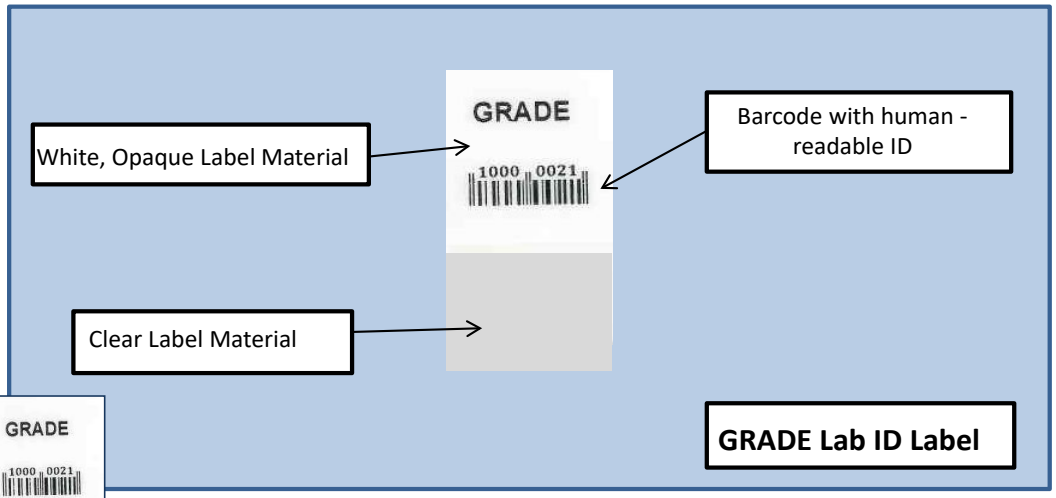
    a. Use collection kit (semi-annual or annual) as needed.
    b. Affix lab ID label from semi-annual (5xxxxxxx series label) or annual (7xxxxxxx) kit to urine cryovial(s).
    c. Affix lab ID label from semi-annual (5xxxxxxx series label) or annual (7xxxxxxx) kit to STF.

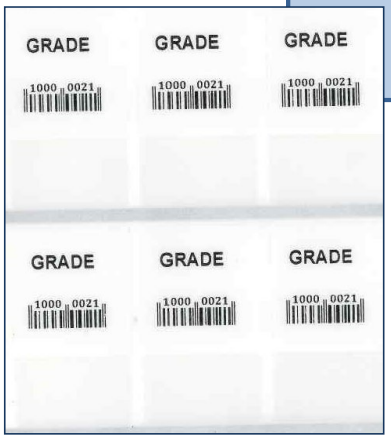
4. Use the small shipper containing a frozen gel pack to ship the capillary sample to the CBL (in the same way a whole blood sample would be packaged/shipped).

    a. Please also return any unused kit components to the CBL, including the postage-paid mailer so that it can be re-used.

5. If collected, the urine sample is shipped per usual protocol: frozen, on dry ice, in the large Styrofoam shipper.

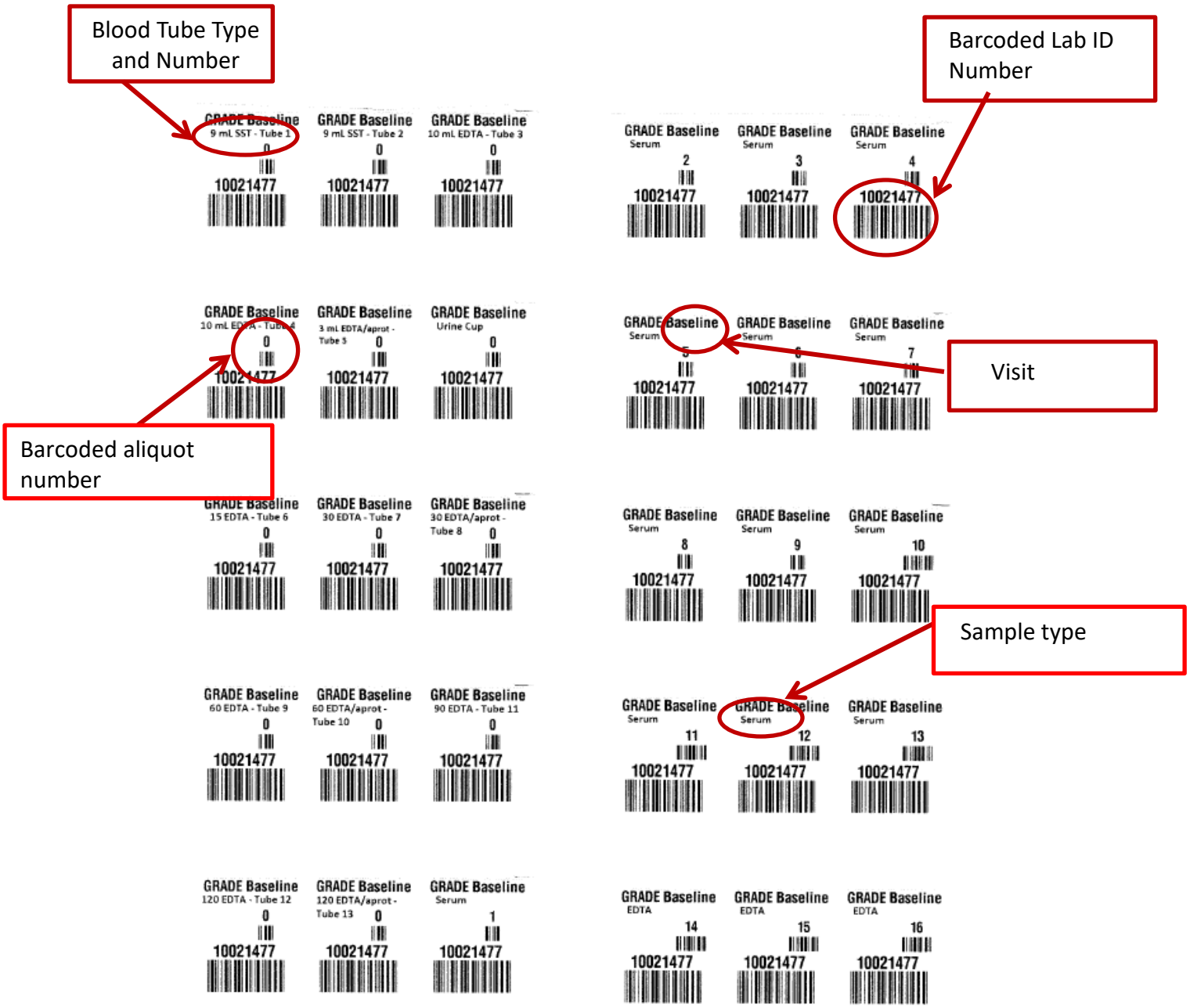
Appendix A. Barcode Labels





When affixing the Barcode label to the cryovial, apply the white portion of the label first with the leading digit of the Barcode number nearest the top of the vial. Then wrap the rest of the label around the vial. The clear part of the label will overlap a portion of the white part of the label without covering any identifying information. The Barcode must be straight up and down from the cap to the foot of the vial in order to be read by a standard barcode reader.

Appendix A. Barcode Labels



Appendix B. Specimen Transmittal Forms

Site ☐☐–☐ Participant ID ☐☐☐☐☐☐ Participant ID Barcode GCode ☐☐☐☐ Sex ☐ M ☐ F

Glycemia Reduction Approaches in Diabetes: A Comparative Effectiveness Study (GRADE)

CENTRAL BIOCHEMISTRY LABORATORY
BASELINE SPECIMEN TRANSMITTAL FORM: FROZEN SAMPLES - BASESTF

This form is used to document collected and shipped frozen specimens to the CBL for the Baseline visit. Refer to the CBL Manual for detailed instructions. Once completed, photocopy this form and retain at the clinic. The original should be placed into a Ziploc bag on top of the polyfoam shipping container. Ship specimens on Monday through Friday (do not ship on Saturdays or days which precede a holiday):

GRADE-CBL/ Advanced Research and Diagnostic Laboratory,
University of Minnesota 420 Delaware Street SE Room L275 Minneapolis, MN 55455-0341
Phone: (612) 273-3645

A. Collection Information

1. Date of collection ☐☐ month ☐☐ day ☐☐☐☐ year

2. Indicate time OGTT fasting sample drawn. Fasting sample should be drawn within 15 minutes before consuming Glucola. If OGTT not performed, indicate time first sample was collected at this visit. ☐☐ hh : ☐☐ mm (24-hour clock)

3. Did participant fast between ≥ 8 and ≤ 18 hours prior to collection? ☐ Yes ☐ No

4. Is the participant randomized to the glargine arm? ☐ Yes ☐ No

Place barcoded Lab ID label here

B. Specimen Collection

Mark (X) on the appropriate box for each sample. Mark 'Processed' if the sample was collected and indicate the number of vials shipped for each specimen, and mark 'Missed' if the sample was scheduled to be collected but missed due to participant refusal or an error.

SPECIMEN - # VIALS EXPECTED *(CAP COLOR)*:	Shipping Temp-erature	Processed	Number of vials shipped	Missed
1. Packed Cell Vial - **2** *(blue-capped)*	Frozen	1		2
2. Urine Cryovials - **9** *(yellow-capped)*	Frozen	1		2
3. Serum Cryovials - **13** *(red-capped)*	Frozen	1		2
4. Plasma Cryovials - **10** *(purple-capped)*	Frozen	1		2
5. Plasma-aprotinin Cryovials - **3** *(orange-capped)*	Frozen	1		2
6. OGTT Plasma 15 minutes- **1** *(purple-capped)*	Frozen	1		2
7. OGTT Plasma 30 minutes- **7** *(4 purple & 3 orange-capped)*	Frozen	1		2
8. OGTT Plasma 60 minutes- **7** *(4 purple & 3 orange-capped)*	Frozen	1		2
9. OGTT Plasma 90 minutes- **1** *(purple-capped)*	Frozen	1		2
10. OGTT Plasma 120 minutes- **7** *(4 purple & 3 orange-capped)*	Frozen	1		2

11. Comments

_____
_____
_____
_____

It is important that the laboratory be able to contact the person who performed this collection and completed this form.

Site name: _____

Site phone #: _____

Site contact: _____

Initials of staff completing form: ☐☐☐

Initials of staff reviewing completed form: ☐☐☐

Form entered in MIDAS? ☐

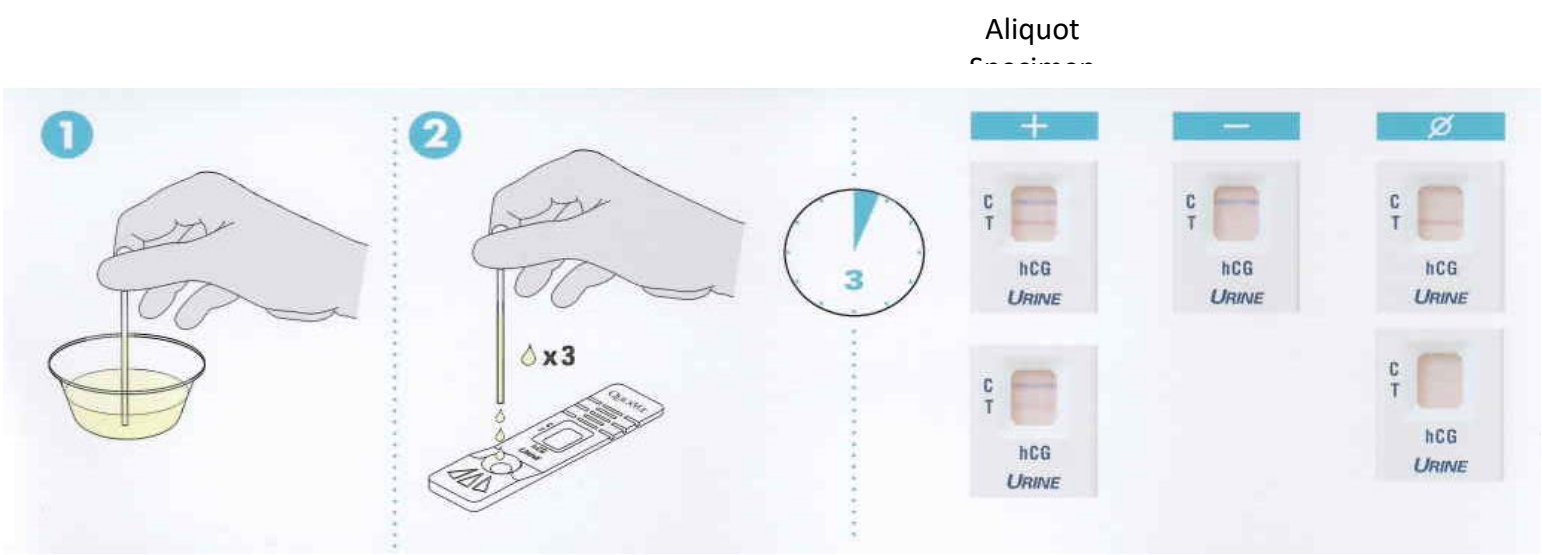
Date form completed (mm/dd/yyyy): ☐☐/☐☐/☐☐☐☐

Appendix C. Pregnancy Test

Test	Tube Name	Fasting Condition	Collection Tube	Visual Reference	Collection/Processing Instruction
Urine Pregnancy Test	Urine Cup	Non-Fasting Acceptable	Urine Cup		• Remove testing device from the test kit and place on a flat, dry surface. Label device with patient identifier. • Holding the dropper from the test kit vertically, transfer 3 full drops of urine to the sample well of the test device and set a timer for 3 minutes. • Interpret the results at exactly 3 minutes.

Result Interpretation:

- **Verify *Internal Quality Control*: You should observe a blue line in the control region (C).**
- POSITIVE RESULT: Two distinct lines appear, one in the control region (C) and one in the test region (T).
- NEGATIVE RESULT: One blue line appears in the control region (C). No apparent red or pink line appears in the test region (T).
- INVALID RESULT: Control line fails to appear. Repeat the test with a new device.

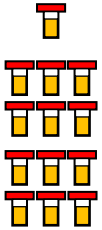
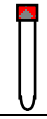
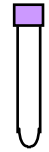
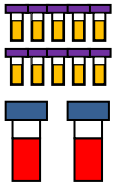
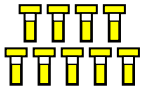


Appendix D. Collection and Processing Flow Diagrams

GRADE Final Run-in Lab Visit

Test	Tube Name	Fasting Condition	Collection Tube	Visual Reference	Aliquot Vials/Caps	Collection/Processing Instruction
Serum Creatinine, eGFR	Tube #1	Non-Fasting Acceptable	2.5 mL red-top with gel separator	Label tube 	Label vial 	• Fill **labeled** tube completely with blood • Invert tube 8 times • Allow tube to sit upright 30-45 min at Room Temperature to clot • Centrifuge tube at 2,000 x g for 15 min • Aliquot 0.5 mL (minimum) serum into **1 labeled** 2-mL cryovial. Tightly fasten with red screw cap • **Refrigerate** cryovial until shipment preparation
HbA1c	Tube #2	Non-Fasting Acceptable	3 mL purple-top EDTA	Label tube 	Do Not Aliquot	• Fill **labeled** tube completely with blood • Invert tube 8 times • **Do not centrifuge this tube!** • **Refrigerate** tube until shipment preparation

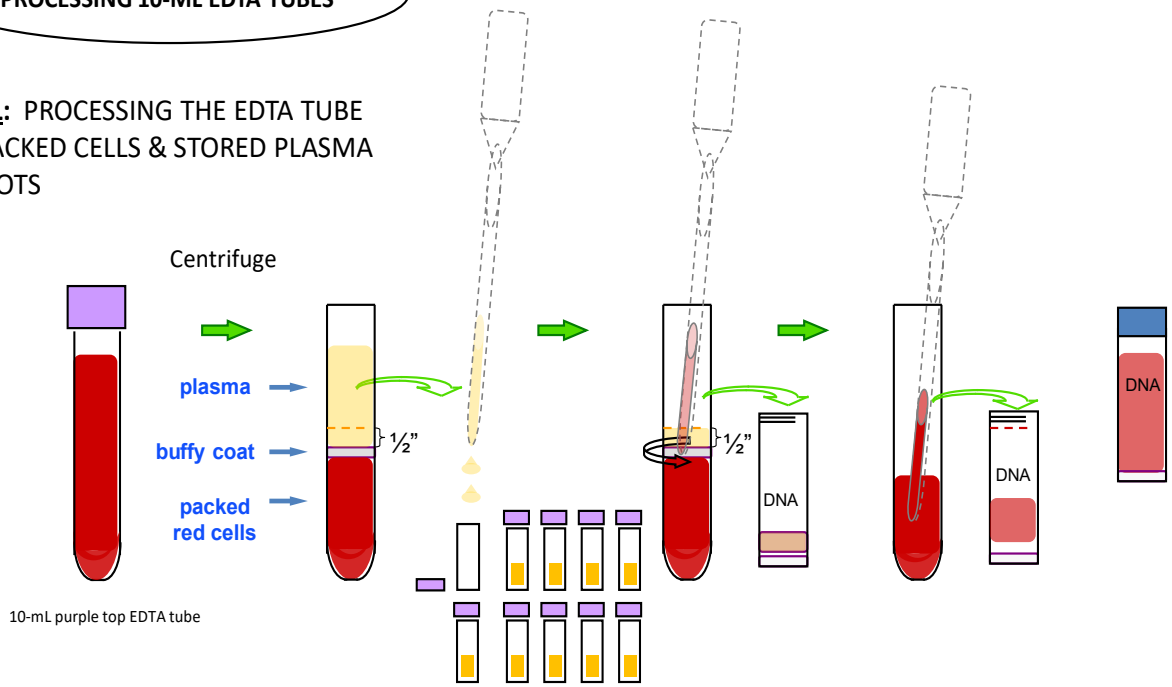
Appendix D. Collection and Processing Flow Diagrams
GRADE Baseline Lab Visit

Test	Tube Name	Fasting Condition	Collection Tube	Visual Reference	Aliquot Vials/Caps	Collection/Processing Instruction
Fasting Lipids Serum Storage	Tube #1	Fasting	9 mL red-top with gel separator	Label tube 	Label vials 	• Fill **labeled** tubes completely with blood • Invert tubes 8 times • Allow tubes to sit upright 30-45 min at Room Temperature to clot • Centrifuge tubes at 2,000 x g for 15 min • Aliquot 1.0 mL serum into **1 labeled** 2-mL cryovial. • Aliquot 0.5 mL serum into the remaining **12 labeled** 2-mL cryovials. • Tightly fasten with red screw caps • Freeze upright at -70°C until shipment preparation
Serum Storage	Tube #2	Fasting	9 mL red-top with gel separator	Label tube 		
Glucose, insulin, c-peptide DNA Plasma Storage	Tube #3	Fasting	10 mL purple-top EDTA	Label tube 	Label vials 	• Fill **labeled** tubes completely with blood • Invert tubes 8 times • Place tube into ice bath immediately • Centrifuge tubes at 2,000 x g for 15 min • Aliquot 0.5 mL plasma into **10** labeled 2-mL cryovials. Tightly fasten with purple screw caps • Aliquot **5mL** packed cells into **2 labeled** 5-mL transport vials and fasten with blue caps (see detailed instructions) • Freeze upright at -70°C until shipment preparation
DNA Plasma Storage	Tube #4	Fasting	10 mL purple-top EDTA	Label tube 		
Plasma Storage	Tube #5	Fasting	3 mL purple-top EDTA + Aprotinin	Label tube 	Label vials 	• Using tuberculin syringe, add 150 µL of aprotinin immediately before blood collection • Fill **labeled** tube completely with blood • Place tube into ice bath immediately • Centrifuge tube at 2,000 x g for 15 min • Aliquot 0.5 mL plasma into **3 labeled** 2-mL cryovials. Tightly fasten with orange screw caps • Freeze upright at -70°C until shipment preparation
Urine Alb:Creat Urine Storage	Urine Cup	Non-Fasting Acceptable	Urine Cup	Label cup 	Label vials 	• Mix urine cup completely • Aliquot 2 mL of urine into **9 labeled** 2-mL cryovials • Tightly fasten yellow screw caps on vials • Freeze upright at -70°C until shipment preparation

Appendix D. Collection and Processing Flow Diagrams
GRADE Baseline Lab Visit

PROCESSING 10-ML EDTA TUBES

DETAIL: PROCESSING THE EDTA TUBE FOR PACKED CELLS & STORED PLASMA ALIQUOTS



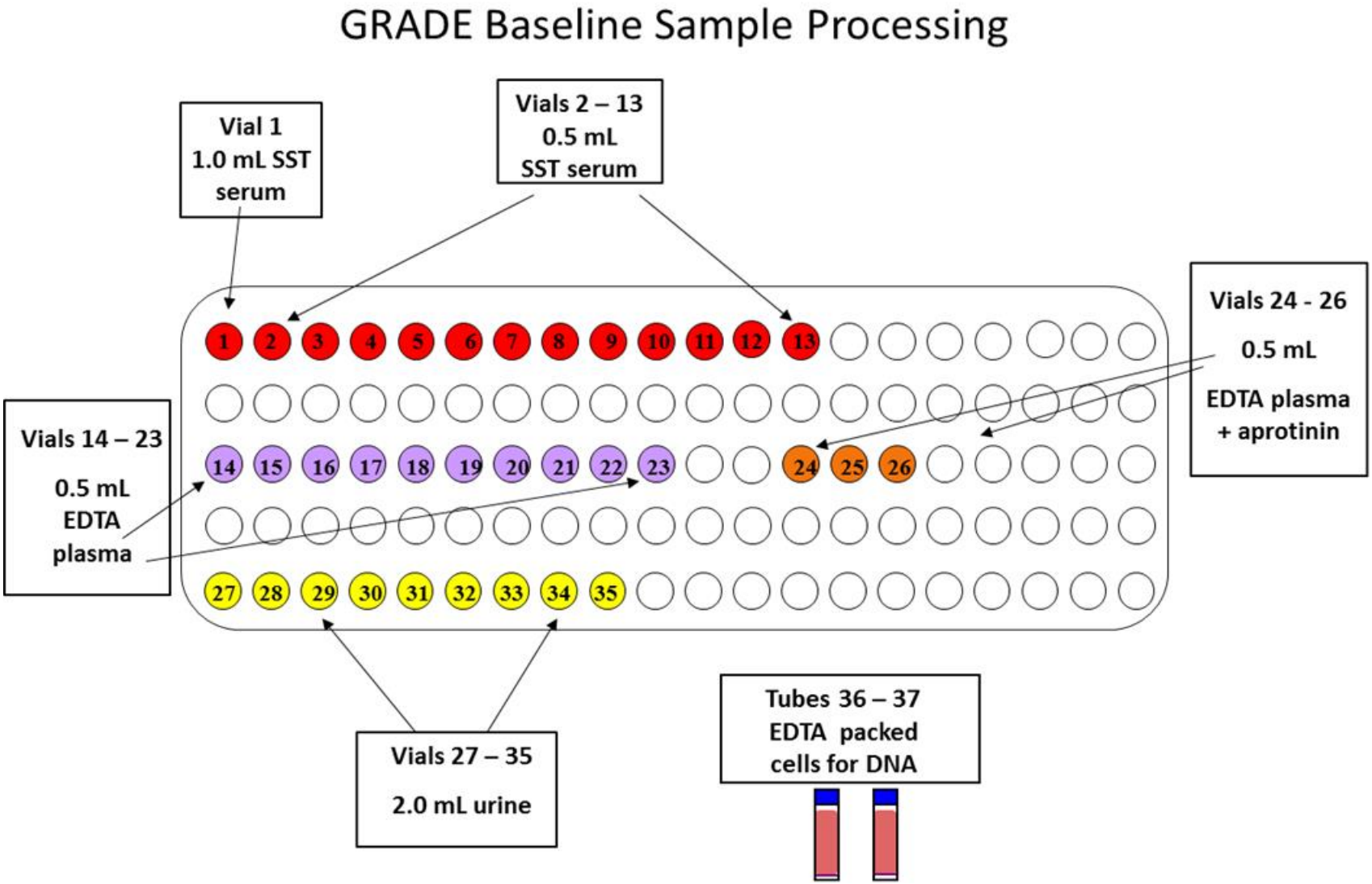
1. Centrifuge the 10-mL EDTA tubes at 2,000 x G for 15 minutes.

2. Taking care not to disturb the cell layer, remove the clear plasma supernatant and transfer 0.5 mL plasma into each of 10 labeled 2-mL cryovials. Aspirate slowly starting at the top of the plasma. Leave a ½ inch layer of plasma above the buffy coat-red blood cell layers. It is important to withdraw only the plasma and none of the buffy coat (containing white blood cells and platelets) that forms at the cell-plasma interface following centrifugation. If some of the buffy coat is accidentally aspirated while removing the plasma, re-centrifuge the tube under the initial processing conditions. Fasten purple screw caps tightly onto the cryovials.

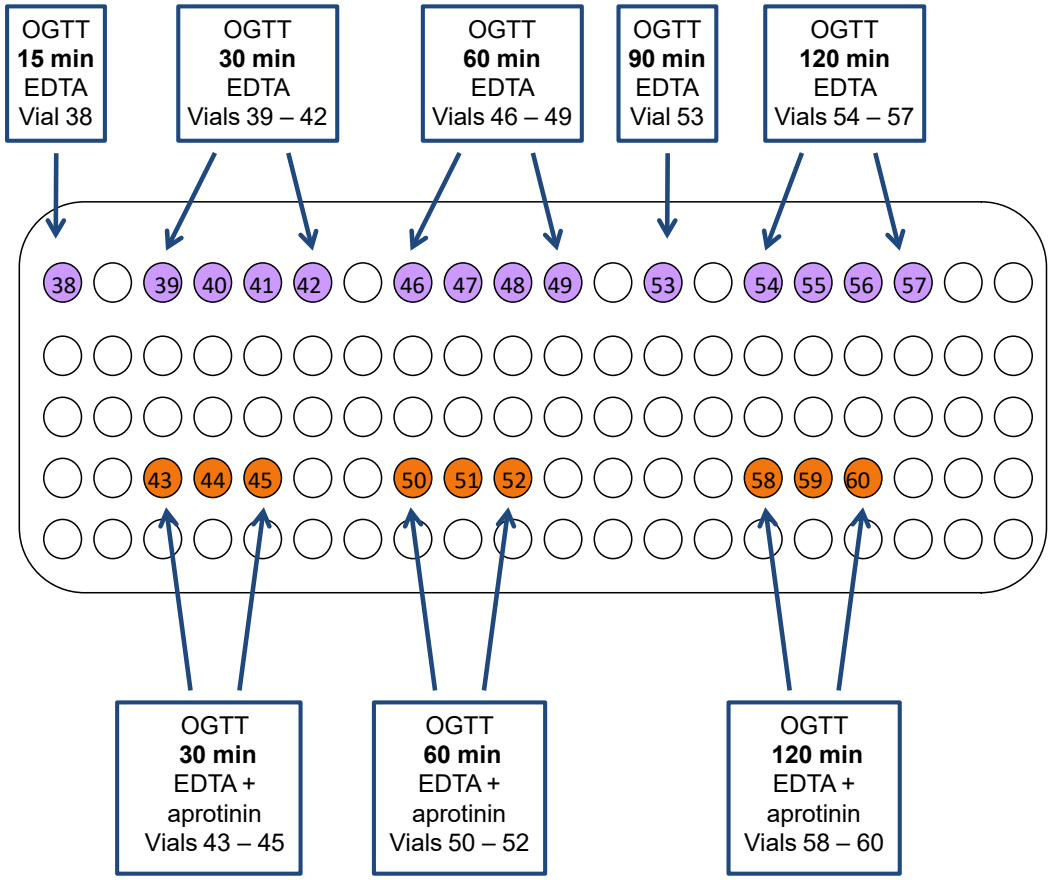
3. Using the same plastic transfer pipet, slowly aspirate the remaining ½" layer of plasma, the buffy coat and *some* of the remaining red cells from the tube. Take care not to aspirate the buffy coat into the bulb of the pipet! 'Ring' the tube with the pipet by carefully aspirating along the wall at the buffy coat layer to ensure maximum transfer. Dispense into the 5-mL 'DNA' vial.

4. Still using the same plastic pipet, transfer all of the remaining packed red cells from the tube into the same 5-mL'DNA' vial. This step will ensure that all of the buffy coat is adequately rinsed from the pipet. Repeat with the second EDTA tube. Fasten blue screw caps tightly on the vials.

Appendix D. Collection and Processing Flow Diagrams
GRADE Baseline Lab Visit



GRADE Baseline OGTT Sample Aliquots



Appendix D. Collection and Processing Flow Diagrams

GRADE Quarterly/Confirmation Lab Visit

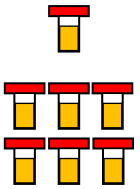
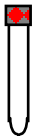
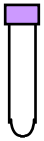
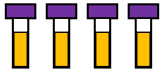
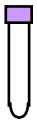
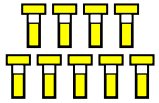
Test	Tube Name	Fasting Condition	Collection Tube	Visual Reference	Aliquot Vials/Caps	Collection/Processing Instruction
HbA1c	Tube #1	Non-Fasting Acceptable	3 mL purple-top EDTA	Label tube 	Do Not Aliquot	• Fill **labeled** tube completely with blood • Invert tube 8 times • **Do not centrifuge this tube!** • **Refrigerate** tube until shipment preparation

Appendix D. Collection and Processing Flow Diagrams

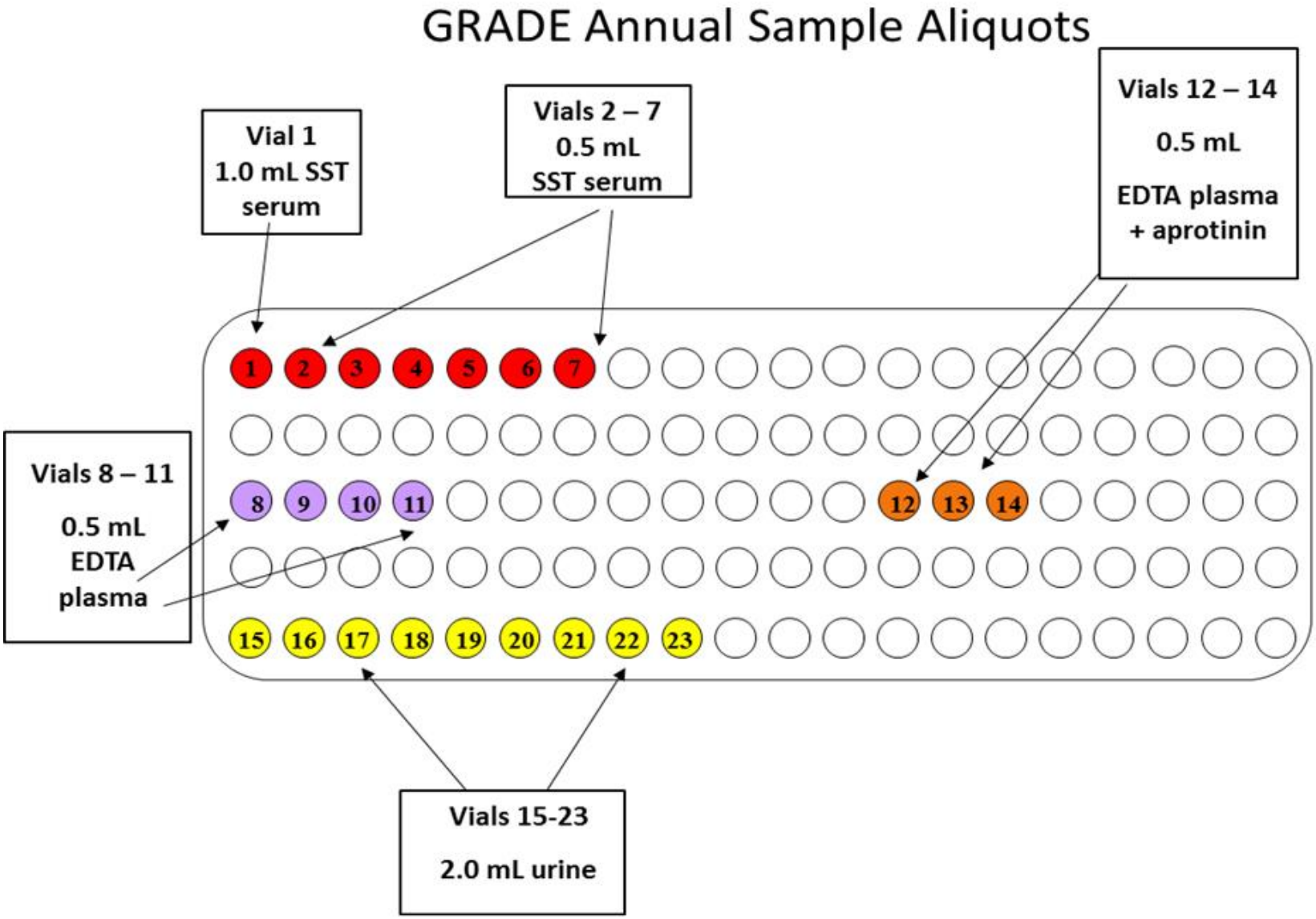
GRADE Semi-Annual Lab Visit

Test	Tube Name	Fasting Condition	Collection Tube	Visual Reference	Aliquot Vials/Caps	Collection/Processing Instruction
HbA1c	Tube #1	Non-Fasting Acceptable	3 mL purple-top EDTA	Label tube 	Do Not Aliquot	• Fill **labeled** tube completely with blood • Invert tube 8 times • **Do not centrifuge this tube!** • **Refrigerate** tube until shipment preparation
Urine Alb:Creat	Urine Cup	Non-Fasting Acceptable	Urine Cup	Label cup 	Label vial 	• Mix urine cup completely • Aliquot 2 mL of urine into **1 labeled** 2-mL cryovials • Tightly fasten yellow screw cap on cryovial • Freeze upright at -70°C until shipment preparation

Appendix D. Collection and Processing Flow Diagrams
GRADE Annual Lab Visit

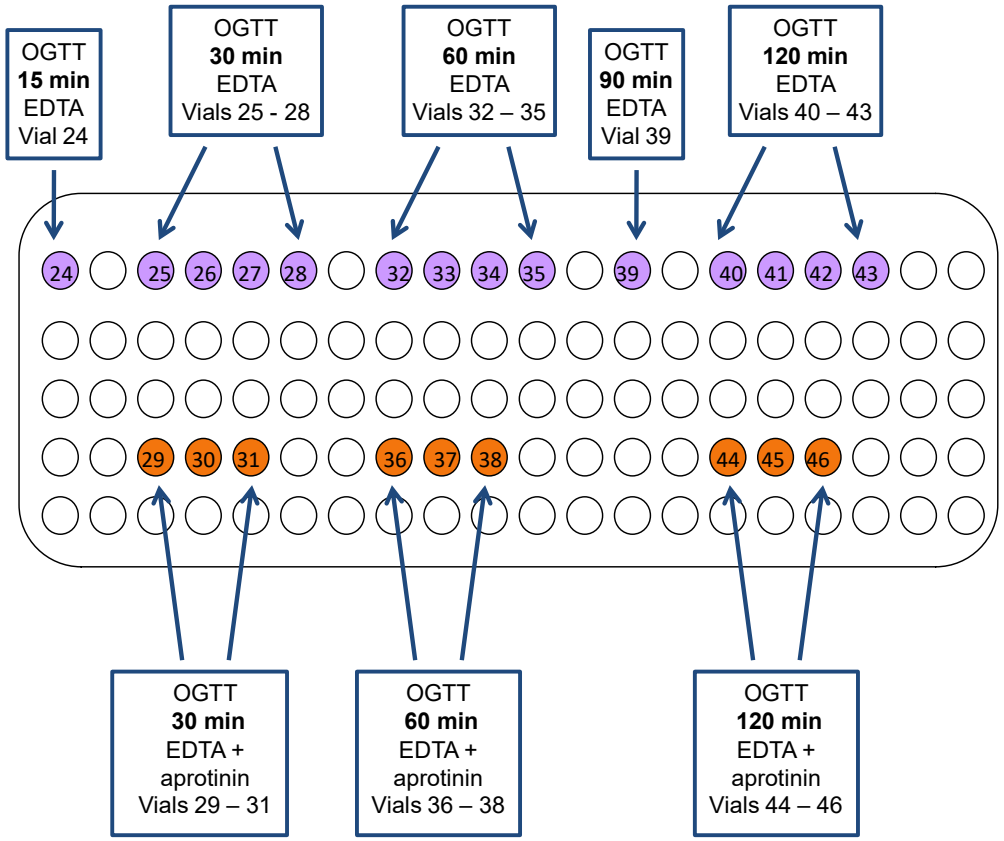
Test	Tube Name	Fasting Condition	Collection Tube	Visual Reference	Aliquot Vials/Caps	Collection/Processing Instruction
Fasting Lipids VB12 (as needed) Serum Creatinine, eGFR	Tube #1	Fasting	2.5 mL red-top with gel separator	Label tube 	Label vials 	• Fill **labeled** tubes completely with blood • Invert tubes 8 times • Allow tubes to sit upright 30-45 min at Room Temperature to clot • Centrifuge tubes at 2,000 x g for 15 min • Aliquot 1.0 mL serum into **1 labeled** 2-mL cryovial • Aliquot 0.5 mL serum into the remaining **6 labeled** 2-mL vials • Tightly fasten with red screw caps • Freeze upright at -70°C until shipment preparation
Serum Storage	Tube #2	Fasting	9 mL red-top with gel separator	Label tube 		
Glucose, insulin, c-peptide Plasma Storage	Tube #3	Fasting	6 mL purple-top EDTA	Label tube 	Label vials 	• Fill **labeled** tubes completely with blood • Invert tubes 8 times • Place tube into ice bath immediately • Centrifuge tubes at 2,000 x g for 15 min • Aliquot 0.5 mL plasma into **4 labeled** 2-mL vials. • Tightly fasten with purple screw caps • Freeze upright at -70°C until shipment preparation
HbA1c	Tube #4	Non-Fasting Acceptable	3 mL purple-top EDTA	Label tube 	Do Not Aliquot	• Fill **labeled** tube completely with blood • Invert tube 8 times • **Do not centrifuge this tube!** • **Refrigerate** tube until shipment preparation
Plasma Storage	Tube #5	Fasting	3 mL purple-top EDTA + Aprotinin	Label tube 	Label vials 	• Using tuberculin syringe, add 150 µL of aprotinin immediately before blood collection • Fill **labeled** tube completely with blood • Place tube into ice bath immediately • Centrifuge tube at 2,000 x g for 15 min • Aliquot 0.5 mL plasma into **3 labeled** 2-mL cryovials. Tightly fasten with orange screw caps • Freeze upright at -70°C until shipment preparation
Urine Alb:Creat Urine Storage	Urine Cup	Non-Fasting Acceptable	Urine Cup	Label cup 	Label vials 	• Mix urine cup completely • Aliquot 2 mL of urine into **9 labeled** 2-mL cryovials • Tightly fasten yellow screw caps on vials • Freeze upright at -70°C until shipment preparation

Appendix D. Collection and Processing Flow Diagrams
GRADE Annual Lab Visit

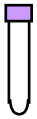
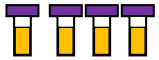


Appendix D. Collection and Processing Flow Diagrams
GRADE Annual OGTT Lab Visit

GRADE Annual OGTT Sample Aliquots



Appendix D. Collection and Processing Flow Diagrams
GRADE OGTT Lab Collection

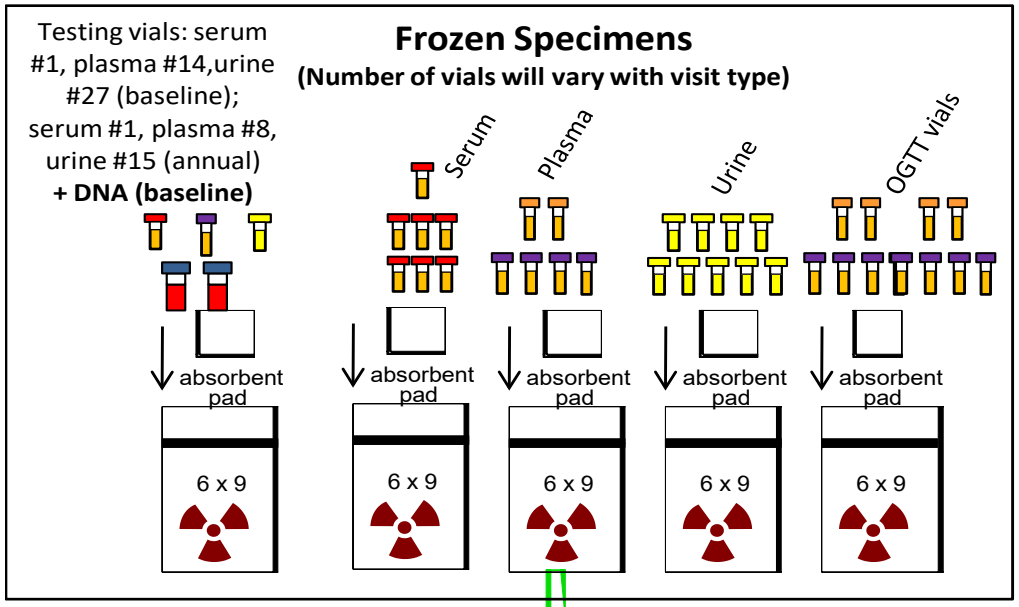
Test	Tube Name	OGTT Timepoint	Collection Tube	Visual Reference	Aliquot Vials/Caps	Collection/Processing Instruction
Glucose, Insulin, C-peptide Plasma Storage	OGTT EDTA	30, 60, 120 min	4 mL purple-top EDTA	Label tube 	Label vial  Plasma	• Fill **labeled** tube completely with blood • Invert tube 8 times • Place tube into ice bath immediately after collection • Centrifuge tube at 2,000 x g for 15 min • Aliquot 0.5 mL (minimum) plasma into **4 labeled** 2-mL cryovials. • Tightly fasten with purple screw caps • Freeze upright at -70°C until shipment preparation
Plasma Storage	OGTT EDTA + aprot	30, 60, 120 min	3 mL purple-top EDTA + aprotinin	Label tube 	Label vial  Plasma	• Using tuberculin syringe, add 150 µL aprotinin immediately before collection • Fill **labeled** tube completely with blood • Invert tube 8 times • Place tube into ice bath immediately after collection • Centrifuge tube at 2,000 x g for 15 min • Aliquot 0.5 mL (minimum) plasma into **3 labeled** 2-mL cryovial. • Tightly fasten with orange screw caps • Freeze upright at -70°C until shipment preparation

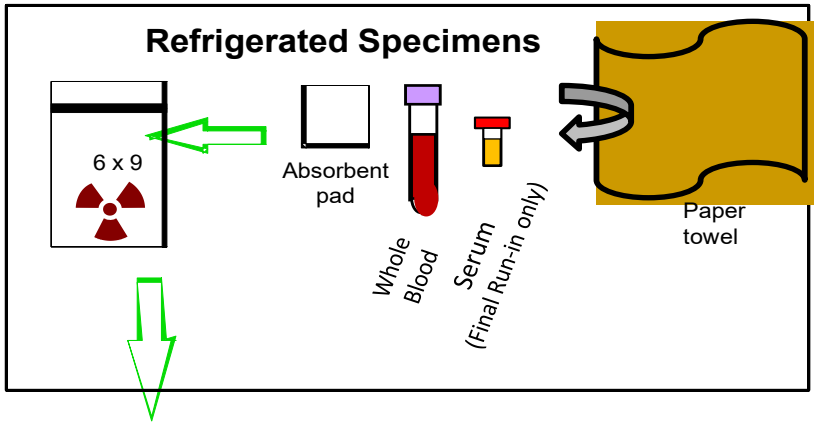
Test	Tube Name	OGTT Timepoint	Collection Tube	Visual Reference	Aliquot Vials/Caps	Collection/Processing Instruction
Glucose, Insulin, C-peptide	OGTT EDTA	15, 90 min	2 mL purple-top EDTA	Label tube 	Label vial  Plasma	• Fill **labeled** tube completely with blood • Invert tube 8 times • Place tube into ice bath immediately after collection • Centrifuge tube at 2,000 x g for 15 min • Aliquot 0.5 mL (minimum) plasma into **1 labeled** 2-mL cryovial. • Tightly fasten with purple screw cap • Freeze upright at -70°C until shipment preparation

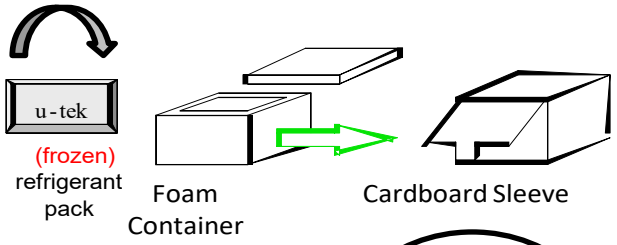
****Centrifuge each timepoint collection set immediately after collection. Do not wait until the end of the 2-hour OGTT test to centrifuge the tubes from all timepoints together****

****Label each vial with the appropriate specimen type/timepoint. Look closely at the labels to ensure accurate identification****

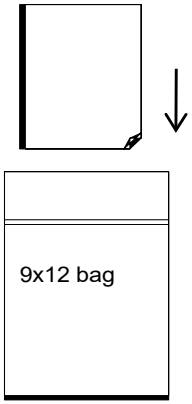
Appendix E. Packaging Specimens for Shipment using 'Dual' Shipping Container

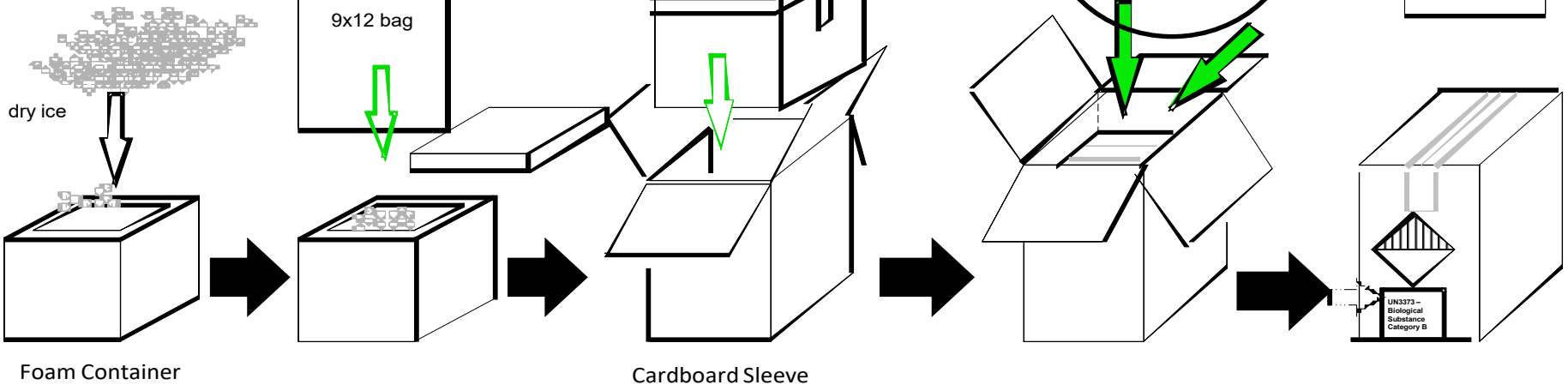




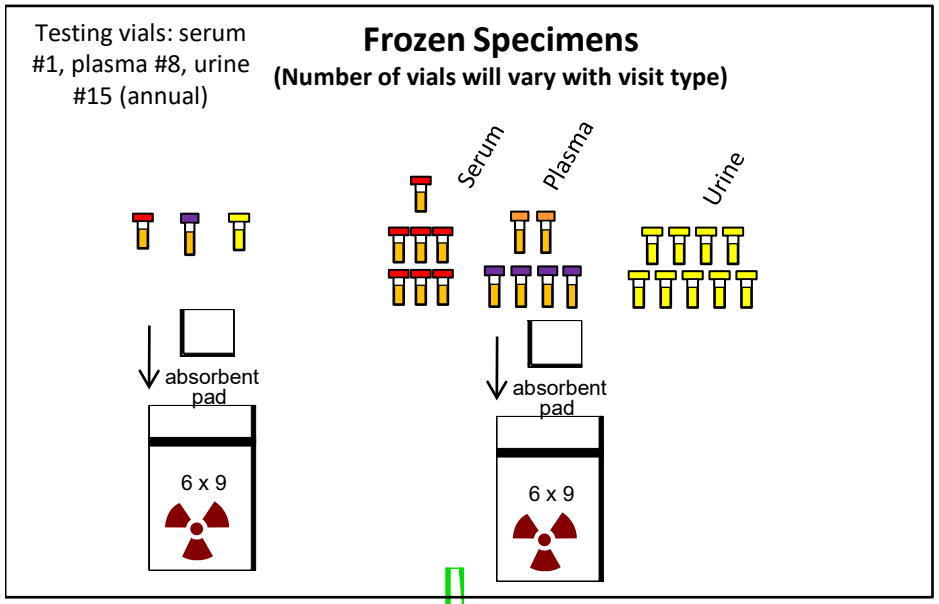


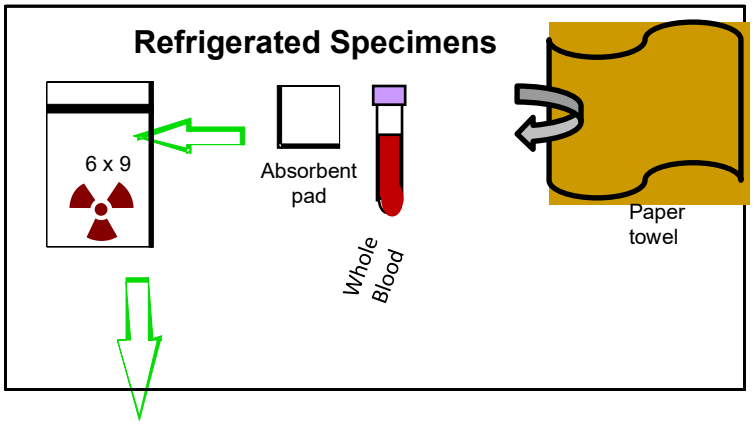
Specimen Transmittal Forms

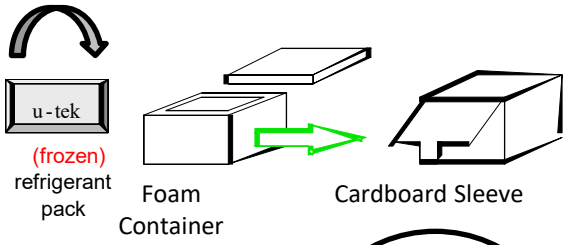




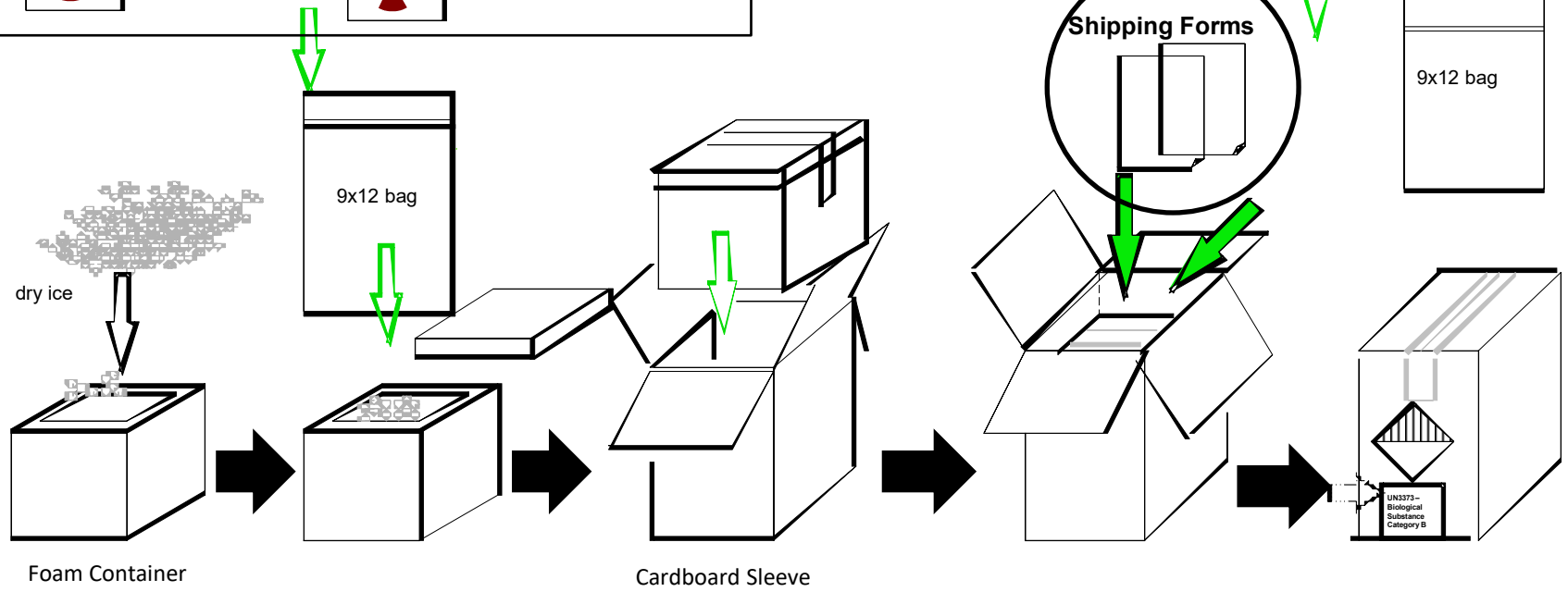
Appendix E. Packaging Specimens for Shipment using 'Dual' Shipping Container *for* Annual visits without OGTT collection



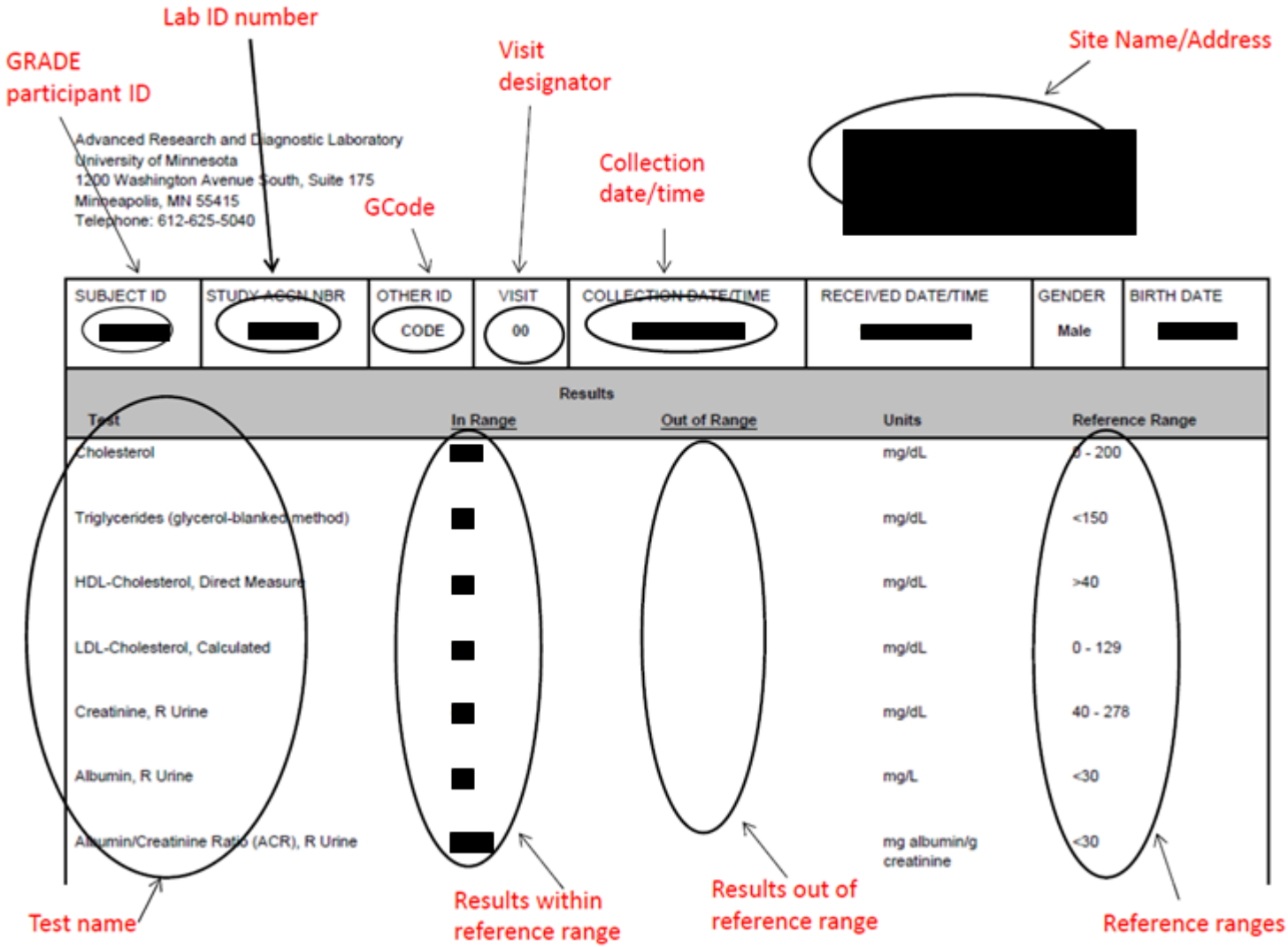




Specimen Transmittal Forms



Appendix F. Results Report



Appendix G. Regulatory requirements for waived testing

OVERVIEW

Every site that performs laboratory testing must follow applicable regulatory requirements. These include federal, state and local requirements for testing as well as requirements for safety and confidentiality of personal information.

WAIVED TESTS

Waived tests include test systems cleared by the Food and Drug Administration (FDA) for home use and those tests approved for waiver under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) criteria. The FDA list of waived tests is continuously being updated. The most current information on FDA-cleared waived tests for verification that the test(s) performed by your laboratory is categorized as waived can be found at the following website: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfClia/testswaived.cfm

CLIA CERTIFICATE OF WAIVER

Before testing patient samples, federal regulations require testing sites to have a CLIA certificate issued by CMS. Sites performing only one or more waived tests must file a Certificate of Waiver application and obtain a separate certificate for each location. A testing site may be covered by the CLIA certificate of the hospital or clinic for which they are associated. The hospital or clinic laboratory manager would be able to provide information on whether the testing site is covered under their CLIA certificate.
To obtain a CLIA Certificate of Waiver, complete CMS form 116 found at: https://www.cms.gov/cmsforms/downloads/cms116.pdf. Your completed CMS form 116 should be sent to the address of the local State Agency for the state in which your laboratory resides. For additional information on how to obtain a Certificate of Waiver, refer to the CMS brochure located online at: http://www.cms.hhs.gov/CLIA/downloads/HowObtainCertificateofWaiver.pdf

Once your site has obtained a CLIA Certificate of Waiver, requirements for testing include:

- Perform only waived tests.
- Follow the current manufacturer's instructions for the waived tests you perform, without any changes.
- Pay the certificate renewal fee every two years.
- Notify your State Agency of any changes in ownership, name, address, or director within 30 days, or if you wish to add tests that are not waived.
- Allow announced or unannounced on-site inspections by a CMS representative.
- Although not routinely done, CMS will inspect waived testing sites under certain circumstances such as:
    - if a complaint has been filed,
    - to determine if the testing site is only performing waived tests,
    - if there is a risk of harm to a patient due to inaccurate testing, and
    - to collect information about practices being used at waived testing sites.

STATE AND LOCAL REQUIREMENTS

State and local jurisdictions vary in how they regulate laboratory testing. Some have requirements governing testing, personnel licensure or phlebotomy. Often there are specific regulations for biohazard safety or the handling and disposal of medical waste. The person overseeing testing should ensure that all state and local requirements are met. When state,

local, and federal requirements are not the same, follow the strictest requirement that applies to your site.

SAFETY

Federal Regulations for Safety

The Occupational Safety and Health Administration (OSHA) requires employers to provide a safe and healthy workplace for employees.
Each site must comply with OSHA standards including:

- Comply with OSHA standards to assure the safety and health of employees. OSHA provides a list of regulations that normally apply to medical and dental offices in a brochure, Medical & Dental Offices— A Guide to Compliance with OSHA Standards: http://www.osha.gov/Publications/osha3187.pdf
- Treat all human blood and certain human body fluids as if they are infectious. Strictly enforce the use of universal precautions and compliance with the bloodborne pathogens standard: http://www.osha.gov/SLTC/bloodbornepathogens/index.html
- Ensure use of safer, engineered needles, sharps containers and personal protective equipment (PPE) such as gloves and protective eyewear. See OSHA's PPE Fact Sheet: http://www.osha.gov/OshDoc/data_General_Facts/ppe-factsheet.pdf
- Implement a sharps injury prevention program. CDC provides a Workbook for Designing, Implementing, and Evaluating a Sharps Injury Prevention Program: http://www.cdc.gov/sharpssafety/pdf/sharpsworkbook_2008.pdf
- Offer hepatitis B vaccination at no cost for employees with possible occupational exposure.
- Provide safety training to employees on handling blood and other infectious materials.
- Provide equipment for safely handling and disposing of biohazardous waste.
- Have a written plan for exposure control. See an example: http://www.osha.gov/SLTC/etools/hospital/hazards/tb/sampleexposurecontrolplan.html
- Maintain records of occupational injuries and illnesses. OSHA provides a record keeping handbook: http://www.osha.gov/recordkeeping/handbook/index.html
- Additional safety practices when performing testing are:
    - No eating, drinking, or applying makeup in areas where samples are collected and where testing is performed.
    - Do not store food in refrigerators where testing supplies or samples are stored.
    - Have sinks for hand-washing or antiseptic hand washing solutions available.
    - Post safety information for employees and patients.
- Development of a site-specific safety plan that describes policies, procedures, and work practices for employee safety provide testing personnel and staff protection from the health hazards that may be involved in testing.
- See Appendix A for an example Safety Plan including an example Safety Training Checklist and Incident Report.

State Regulations for Safety

Many states have Occupational Safety and Health (OSH) Plans that are monitored by OSHA. If you live in a state that provides a State Plan, you must comply with the State OSH standards. OSHA provides information on State OSH programs: http://www.osha.gov/dcsp/osp/index.html

Appendix G. Regulatory requirements for waived testing – Quiz

GRADE Urine Pregnancy Competency Test

Name:_____

Date:_____

1. How many drops of urine are placed in the sample well:
    a) 1
    b) 2
    c) 3
    d) 4
2. Results should be interpreted after how many minutes?
    a) 2
    b) 3
    c) 4
    d) 5
3. T or F A patient identifier should be recorded on the test device.
4. T or F A blue line in the C (control) region indicates the test device is working appropriately.
5. T or F Two distinct lines, one in the C (control) region and one in the T (test) region indicate a positive pregnancy result.
6. T or F Two potential causes of falsely negative urine pregnancy test are very dilute urine and when the level of hCG is below the sensitivity level of the test.
7. T or F I have reviewed the package insert and am competent to perform testing.

Appendix G. Regulatory requirements for waived testing – Quiz

Answers:
1=C
2=B
3=T
4=T
5=T
6=T
7=T

Appendix G. Regulatory requirements for waived testing - Pregnancy Result Form

GRADE **Quidel QuickVue Pregnancy Screen for Urine hCG**

Date	Participant Name/Study ID#	Pregnancy Result (Report as POS or NEG)	Pregnancy Kit Lot Number	Blue line in (C) region indicating internal control acceptable (circle one)	Staff Initials
				yes no	
				yes no	
				yes no	
				yes no	
				yes no	
				yes no	
				yes no	

Appendix G. Regulatory requirements for waived testing - Pregnancy Test External Quality Control Result Form

Quidel QuickVue hCG External Control
***Perform external controls on all new shipments of test devices**

Date of kit receipt	Kit lot number	Control lot number	Control expiration date	Result--positive control	Result--negative control	Date of control testing	Staff initials

Appendix H. GRADE CBL Tests and Reference Ranges

Laboratory Test	Reference Range	Units
HbA1c	4.3 – 6.0	**%**
Lipid Panel		
Cholesterol	< 200	mg/dL
Triglycerides	< 150	mg/dL
High Density Lipoprotein (HDL)	female >50; male >40	mg/dL
Low Density Lipoprotein (LDL), calculated	< 129	mg/dL
Creatinine, Serum	female 0.4 – 1.1; male 0.5 – 1.2	mg/dL
C – peptide	0.37 – 1.47	nmol/L
Glucose	60 – 99	mg/dL
Insulin	12 – 150	pmol/L
Urine Albumin **Urine Creatinine**	< 30 mg albumin / g creatinine *for calculated albumin:creatinine ratio (ACR)*	mg albumin / g creatinine
Estimate Glomerular Filtration Rate (eGFR)	> 60	mL/min/1.73m^2
Vitamin B12	≥400	pg/mL

Tests by Visit

Final Run-in
HbA1c
Creatinine, serum
Estimated Glomerular Filtration Rate (eGFR)

Baseline
Lipid Panel
Urine Albumin:Creatinine ratio (ACR)
DNA Extraction
OGTT – C-peptide, glucose, insulin x 6 timepoints

Quarterly
HbA1c
Red cell volume (for CGM visits)
Glycated albumin (for CGM visits)

Semi-Annual
HbA1c
Urine Albumin:Creatinine ratio (ACR)
Vitamin B12 (selected visits)
Red cell volume (CBC) (for CGM visits)
Glycated albumin (for CGM visits)

Annual
HbA1c
Creatinine, serum
Estimated Glomerular Filtration Rate (eGFR)
Lipid Panel
OGTT – C-peptide, glucose, insulin x 6 timepoints
Urine Albumin:Creatinine ratio (ACR)
Vitamin B12 (selected visits)
Red cell volume (CBC) (for CGM visits)
Glycated albumin (for CGM visits)

Appendix I. Guidelines for the Recollection of GRADE Biospecimens and Collection of Interim Visit Samples

In certain situations, some biospecimens may need to be recollected from a participant. The CBL or Coordinating Center may contact a GRADE clinical site with a request to recollect a biospecimen. Alternatively, a site may have difficulty obtaining a biospecimen at a regularly scheduled visit and may arrange to collect the biospecimen at a later time. Lastly, a site may re-test samples at final run-in per the guidelines outlined in the GRADE Manual of Procedures (see GRADE MOP sections 5.4.10-5.4.12).

Interim visits are those between scheduled visits and may be conducted for the monitoring of participant safety or outcomes. The following guidelines describe how to collect the samples and document the collection of interim visit samples.

Please contact the CBL before recollecting any samples. If the participant agrees to a recollection or collection of a sample that you were unable to obtain at a previously scheduled visit, the following guidelines will help with the recollection of biospecimens.

Summary table of recollections and interim collections by visit

Visit type	Purpose	STF	Collection kit	Lab ID number format
Final run-in HbA1c retest	HbA1c did not meet randomization criteria; retest HbA1c only	REDRAW	Final run-in	2xxxxxxx
Re-screening (F2, etc)	participant not randomized; repeat all screening and run-in activities	FRISTF	Final run-in	2xxxxxxx
OGTT (re)collection	OGTT samples were not collected or were collected separate from visit	REDRAW	Baseline or annual, as appropriate	1xxxxxxx (baseline) or 7xxxxxxx (annual)
eGFR repeat	eGFR requires monitoring	REDRAW	Annual	7xxxxxxx
Confirm HbA1c >9%	HbA1c was over 9.0%; site needs to confirm	CONFIRM9	Quarterly/ confirmation	3xxxxxxx

1. RECOLLECTION GENERAL GUIDELINES

1.1. Clinical sites will be contacted directly by the CBL if a specimen recollection is necessary. Only recollect the biospecimen(s) instructed by the CBL; note that **not** all biospecimens from a visit may need to be recollected. **Timelines for recollection of specimens by visit:**
If the CBL or Coordinating Center contacts your site and requests:

- Recollection of Final Run-in Visit labs:
  - Recollect biospecimens as soon as possible after initial collection. Recollection of biospecimens and review of results must be completed at least 5 business days before the baseline visit occurs.
- Recollection of Baseline Randomization Visit labs:
  - Recollect biospecimens (except packed cells for DNA extraction and OGTT samples) within 5 days of the baseline visit.
- Recollection of Quarterly, Semi-Annual or Annual Visit labs:

- Recollect biospecimens as soon as possible after scheduled visit, preferably within 5 days of the visit. Refer to Section 1.2 below for specific instructions on recollection of OGTT biospecimens.

- Recollection of DNA:
  - If DNA is the only sample requested for recollection by the CBL, then recollect packed cells for DNA extraction at the participant's next scheduled visit. Send both the REDRAW collection form (for the DNA extraction) and the scheduled visit form with biospecimen shipment. Refer to Section 1.3 below for specific instructions on recollection of DNA biospecimens.
- Repeat eGFR testing:
  - If, at an Annual or Interim visit, the eGFR is <30 ml/min/1.73m^2, <u>metformin must be discontinued</u>. There is no provision for repeat testing to 'confirm' this value.
  - If, at an Annual or Interim visit, the eGFR is 30 – 45 ml/ min/1.73m^2, metformin may be continued with caution. PI and site staff judgement must be used to determine if more frequent monitoring of eGFR is desired. Any samples for interim eGFR monitoring should be sent to the CBL. See section 1.4 below for specific instructions on collection of samples for this eGFR monitoring.
- Confirm an HbA1c value is >9.0%:
  - Refer to *Section 8.1 Diabetes Care* in the GRADE study MOP for guidelines in determining the necessity of a confirmation HbA1c visit. This visit is conducted 3 to 6 weeks following the triggering HbA1c value.

1.2. OGTT Recollection:

<u>NOTE: if OGTT is being recollected because participant was not fasting, see also Appendix L for instructions on recollecting all fasting portions of visit.</u>

<u>If only the OGTT portion of an annual visit is to be recollected, the **fasting** EDTA plasma sample must also be recollected.</u> Use a 2mL purple-top EDTA blood collection tube for collection of the fasting sample. You will need to use one of the tubes from the supply of extra tubes you keep on hand or request additional supplies from the CBL. To collect the fasting EDTA tube:

- Fill **labeled** 2mL tube completely with blood.
- Invert tube 8 times.
- Place tube into ice bath immediately.
- Centrifuge tube at 2000 x g for 15 minutes.
- Aliquot 0.5mL of plasma into one **labeled** 2mL vial.
- Tightly fasten with purple screw cap.
- Freeze upright at -70^{o}C until shipment preparation.
- Collect the other OGTT timepoints as described in the CBL MOP.
- Package and ship frozen on dry ice as described in the CBL MOP.
- Document collection on REDRAW STF.
  - Record visit number for the visit at which the OGTT **should** have occurred. Do <u>not</u> record the visit number as the visit at which the collection is actually taking place.

1.3. Recollections of Packed Cells for DNA:

- Request a DNA recollection kit from the CBL.
- The participant does not need to be fasting.
- Refer to Collection and Processing Flow Diagram in Appendix D of the CBL MOP as needed
- Using a DNA recollection kit, prepare two 5-mL blue-cap 'DNA' vials labeled with aliquot numbers 36-37.

- Collect two (2) 10mL EDTA tubes. Perform the venipuncture filling each blood tube as completely as possible (i.e., until the vacuum is exhausted and blood flow ceases). After the tube is filled with blood, gently invert 8 times and place upright into a crushed **ice bath** (cup or beaker containing wet ice) OR centrifuge immediately.
- Remove the purple stopper EDTA tubes from the ice bath and place into a centrifuge trunnion. Balance the centrifuge trunnions, then centrifuge at 2,000 x g for 15 minutes.
- Match the Lab ID from the 10mL EDTA tubes to the Lab ID labels that appear on the 5-mL DNA transport vials labeled with aliquots #36 – 37.
- Taking care not to disturb the cell layer, remove the clear plasma supernatant and **discard**. Leave a ½ inch layer of plasma above the buffy coat-red blood cell layers. It is important to withdraw only the plasma and none of the buffy coat (containing white blood cells and platelets) that forms at the cell-plasma interface following centrifugation. If some of the buffy coat is accidentally aspirated while removing the plasma, return the buffy coat and plasma to the original tube and re-centrifuge the tube.
- Using the same plastic pipette, slowly aspirate the remaining ½ inch layer of plasma, the buffy coat, and some of the remaining red cells from the tube. Take care not to aspirate the buffy coat into the bulb of the pipette. "Ring" the tube with the pipette by carefully aspirating along the wall at the buffy coat layer to ensure maximum transfer. Dispense into the 5-mL DNA transport vial (#36).
- Still using the same pipette, go back and transfer all of the remaining packed red cells from the tube to the same 5-mL DNA vial. This step will ensure that the entire buffy coat is adequately rinsed from the pipette.
- Repeat with the second 10mL EDTA tube into aliquot #37. Fasten **blue** caps onto the 5-mL DNA transport vials.
- Re-stopper the empty blood collection tubes and discard.
- Freeze upright at -70^{o}C until shipment preparation.
- Package and ship frozen on dry ice as described in the CBL MOP.
- Document collection on the REDRAW STF.
  - Record visit number as 00 (baseline, the visit at which the collection **should** have occurred).

1.4. Repeat Creatinine / eGFR Collection:

- Repeat creatinine testing and eGFR calculation to monitor participant when eGFR is 30 – 45 ml/min/1.73 m^2 *at Annual Visits only:*
  - The repeat testing will be done at a visit being conducted for safety monitoring of participants who may be a high risk for developing an eGFR <30 ml/min/1.73m^2.
  - Use a new annual visit collection kit to collect the 2.5mL red-top with gel separator tube only. Process and ship the 1mL aliquot of serum as directed in the CBL MOP for the Annual visit (i.e. store and ship the sample at frozen temperature).
  - Complete the REDRAW STF for this collection. In section A (Collection information), for Question 2 (Visit), record the number of the most recent previous annual visit; Question 6 (Reason for redraw), check 'Other.' **In the comments section, write "monitor eGFR."**
- Repeat due to *request by CBL only* (e.g. poor specimen, lab incident, etc.)
  - If CBL requests a repeat draw of serum from the from the Final Run-in visit for repeat eGFR testing, use a new Final Run-in collection kit to collect the 2.5mL red-top with gel separator tube only. Process and ship the 1mL aliquot of serum as directed in the CBL MOP for the Final Run-in visit (i.e. store and ship the sample at refrigerated temperature).
  - Complete the REDRAW STF for this collection. In section A (Collection information), Question 2 (Visit), record the visit number at which the initial sample was obtained and the repeat is being requested; for Question 6 (Reason for redraw), check 'initial draw done but could not be tested'.

1.5. Confirmation of HbA1c >9.0%

- Collect 3-6 weeks following triggering HbA1c value:
    - Use a quarterly/confirmation kit to collect a 3mL EDTA purple-top blood tube.
    - Do not centrifuge this tube. Keep tube upright at refrigerated temperature (2-8ºC) until shipping.
    - Package and ship according to the instructions for quarterly visits.
    - For in-clinic samples collected via a venipuncture, document collection on the CONFIRM9 STF.
        - You will record the visit number of the initial HbA1c > 9%. The visit number for this collection ("IN") is hard-code printed at the top of the form.
        - If a blood sample for this confirmation visit is collected via an at-home capillary collection, refer to Section 11 for procedure.

1.6. Labeling Instructions for Recollections: Use the appropriate visit collection kit containing a **new** set of Lab ID labels; affix labels as needed to the collection tubes and aliquot vials. **Discard** any remaining unused labels. Any remaining collection tubes, aliquot vials and caps, and other items may be kept to use as 'extras.'

1.7. Required Documentation for Recollections: Use the REDRAW STF in the As Needed Forms folder on the GRADE website. Record the two-digit visit code as directed in the Summary table of recollections by visit, above, for each recollection situation. For example, if recollecting the serum from the Final Run-in Visit, use the visit code 'F1' (or 'F2' for rescreened participants) on the REDRAW STF. Use '00' for DNA recollections. Use 'IN' for eGFR monitoring after annual visit.

Read the STF carefully to identify the appropriate shipping temperature(s) for the recollected biospecimen(s).

1.8. Results for Recollected Biospecimens: If the recollection is for a biospecimen testing vial (not a storage vial), the testing will occur and the results report will print according to the same schedule as a regular visit.

2. RECOLLECTION FOR RE-TESTING AND RE-SCREENING

A re-test refers to the recollection of a biospecimen in order to repeat a particular test because there is reason to believe that the result will be different than the original test result. Staff should **only** collect the biospecimen(s) required for the assay that is being repeated. The Redraw Specimen Transmittal Form is completed to document this collection.

A re-screen involves the completion of all screening and run-in activities in their entirety. When a participant is re-screened, all biospecimens are collected again using the appropriate Specimen Transmittal Form(s).

See GRADE Study MOP sections 5.4.10 – 5.4.12 for circumstances when collection of final run-in samples for re-testing and participants who have been re-screened is appropriate. These guidelines only apply to testing performed at the CBL on final run-in visit collections.

2.1. <u>Recollecting Samples for Re-test at final-run-in:</u>

1) Use a new final run-in collection kit.
    a. For HbA1c re-tests

i. Collect only the 3mL purple-top tube. Do NOT collect the serum sample. Remaining supplies from the kit (2.5mL serum collection tube, cryovial, cap, pipet) may be saved with other 'extra' supplies. **Discard** any remaining unused labels.
ii. Complete the REDRAW STF. For question 2, Visit, record 'F1.' For question 6, Reason for redraw, check the box 'Retest HbA1c.'

Note: if the re-test is for a participant who has been re-screened and has had a second final run-in visit, record 'F2' as the visit, 'F3' for a third final run-in visit, etc.

b. For creatinine/eGFR re-tests
i. Collect only the 2.5mL red-top tube. Do NOT collect the whole blood EDTA sample. Remaining supplies from the kit (3mL EDTA collection tube) may be saved with other 'extra' supplies. **Discard** any remaining unused labels.
ii. Complete the REDRAW STF. For question 2, Visit, record 'F1.' For question 6, Reason for redraw, check the box 'Other,' record in the Comments box 'creatinine/eGFR re-test.'

Note: if the re-test is for a participant who has been re-screened and has had a second final run-in visit, record 'F2' as the visit, 'F3' for a third final run-in visit, etc.

2) Ship sample to CBL at refrigerated temperature, as for usual final run-in visit.
3) Results will be reported on the same schedule as a final run-in visit.

2.2. <u>Recollecting Samples for Re-screened Participants at final run-in:</u>

1) Use a new final run-in collection kit. Collect both the whole blood tube and serum tube. Process samples as directed in CBL MOP.
2) Complete the FRISTF. For question 3, Visit, record 'F2' for the first re-screen attempt and 'F3' for the next etc.
3) Ship samples to CBL at refrigerated temperature, as directed for a final run-in visit.
4) Results will be reported on the same schedule as a final run-in visit.

Appendix J. Guidelines for the Collection of Missed Biospecimens

A sample is 'missed' when it was not or could not be collected at the participant's regularly scheduled visit. **If a sample has been missed, contact the Coordinating Center before scheduling a time to collect the sample.** After the Coordinating Center has been consulted, the collection may be done as an unscheduled visit or at participant's next scheduled visit.

COLLECTION OF MISSED BIOSPECIMENS AT AN UNSCHEDULED VISIT

1. Use collection kit/lab ID labels corresponding to visit of the missed collection. For example, if urine was not collected at a semi-annual visit, use a new semi-annual collection kit to obtain the sample. After collection, discard any unused lab ID labels; store remainder of any unused kit contents as 'extra' supplies.
2. Use the REDRAW STF to document collection of the missed sample.
    a. Record visit code on STF as the visit at which the sample should have been obtained.
3. Ship sample to CBL as for usual for the visit, either at refrigerated or frozen temperature.
4. Results will be reported on the same schedule as usual for the visit.

COLLECTION OF MISSED BIOSPECIMENS AT PARTICIPANT'S NEXT SCHEDULED VISIT

1. Collection biospecimens for the *scheduled* portion of the visit
    a. Collect the scheduled visit's samples using appropriate collection kit/lab ID labels.
    b. Document the scheduled visit's collection on appropriate STF.
    c. Record visit code of the scheduled visit on STF.
2. For the collection of the *missed* biospecimens from the previous visit where the sample was *missed*
    a. Collect the missed visit's samples using collection kit/lab ID labels corresponding to visit of the missed collection. For example, if urine was not collected at a semi-annual visit, use a new semi-annual collection kit to obtain the sample. After collection, discard any unused lab ID labels; store remainder of any unused kit contents as 'extra' supplies.
    b. Document the missed visit's collection on the REDRAW STF.
    c. Record visit code of the missed visit on STF.
3. Package and ship samples to CBL as for usual for the visit, either at refrigerated or frozen temperature.
    a. You will be sending 2 STFs (Visit STF and REDRAW) and samples with 2 different lab IDs.
4. Results will be reported on the same schedule as usual for the visits.

Appendix K. Guidelines for Collection of Emotional Distress Substudy (EDS) Biospecimens

This appendix describes the collection of biospecimens for the Emotional Distress Substudy (EDS). Participants in the EDS will have EDS collections at their baseline, semi-annual and annual GRADE visits. The EDS biospecimen will be collected as part of the regular GRADE biospecimen collection at the baseline GRADE visit and annual visit using the current baseline and annual collection kits. i.e NO separate blood collections will be required for EDS at baseline and annual visits. However, an additional biospecimen for EDS will be collected at the semi-annual visit, using a separate EDS collection kit. Please refer to the table below for a summary of EDS biospecimen collection by visit.

Summary Table of EDS biospecimen collection by visit

EDS visit	Additional collection required?	Collection kit(s) used	STFs completed	Test to perform (at CBL)
Final run-in*	NO*	Final run-in	FRISTF; EDSSTF	hsCRP
Baseline	NO	Baseline	BASESTF; EDSSTF	hsCRP
Semi-annual	YES	Semi-annual; 'EDS or VB12' semi-annual	QTSMISTF; EDSSTF	hsCRP
Annual	NO	Annual	ANNSTF; EDSSTF	hsCRP

* Only in certain situations will sample be collected at the final run-in visit. No additional sample collection is required at the final run-in visit; however an additional serum aliquot will be prepared from the serum tube already collected.

1. Preparation

1.1. The participant will be fasting for the baseline and annual visit collections, but fasting is not required at the final run-in or semi-annual visit.

1.2. The CBL will provide a separate EDS collection kit for collection of a serum tube at the semi-annual visit. This EDS semi-annual collection kit can be requested via the online supply re-order website. No separate collection kit is required for an EDS visit at the final run-in/baseline and annual visits. If the sample is collected at the final run-in visit rather than at the baseline visit, an extra aliquot of serum is prepared from the SST tube already collected at final run-in (see section 6 below for details).

1.2.1. **NOTE**: The kit previously referred to as the "EDS semi-annual" collection kit has been renamed as the "EDS or VB12 semi-annual" collection kit. The "EDS or VB12 semi-annual" kit may be used in either of 3 situations:

1.2.1.1. A semi-annual visit where only an EDS collection will take place

1.2.1.2. A semi-annual visit where only a vitamin B12 collection will take place. See Appendix O for more information about collections for vitamin B12 measurements.

1.2.1.3. A semi-annual visit where both an EDS and vitamin B12 collection will take place. See Appendix O for more information about collections for vitamin B12 measurements.

1.2.2. Do not worry if there are concerns that the EDS testing will take place when only the vitamin B12 testing is desired, or vice versa. **As long as the correct STF accompanies the sample the correct testing will occur.**

1.3. **The EDSSTF should be completed for all EDS visits, regardless of whether or not a separate collection is done.**

2. Organization

2.1. Prior to the patient's semi-annual EDS visit, remove the sheaf of barcoded Lab ID labels and the blood collection tube from the 'EDS or VB12' collection kit.

2.2. Affix one 'EDS or VB12' kit Lab ID label to the SST blood collection tube. Once blood processing is complete and the EDS specimen transmittal form has been completed, discard any remaining Lab ID labels from the EDS kit.

2.3. Arrange the set of blood collection tubes in a rack in *the order in which they will be collected, as follows:*

EDS SEMI-ANNUAL VISIT
Tube #1 red stopper SST tube *(provided with 'EDS or VB12' collection kit)*
Tube #2 3-mL purple stopper EDTA tube *(provided with GRADE semi-annual collection kit)*
One urine container *(provided with GRADE semi-annual collection kit)*

**Note: the lab ID label on tube #1 (SST) will be different from the lab ID labels on tube #2 (EDTA) and the urine container. Use extra care when collecting EDS visit samples to ensure that the participant's samples are collected and labeled appropriately.

2.4 Prepare a tray of the cryovials and color-coded screw caps that will contain the final samples to be shipped to the CBL. **The Lab ID labels on these vials must match those affixed to the corresponding set of blood collection tubes.**

Affix Lab ID labels to all of the cryovials in the kit prior to blood collection.

EDS SEMI-ANNUAL VISIT
a. One 2-mL cryovial with red screw cap *(provided with 'EDS or VB12' collection kit)*
b. One 2-mL cryovial with yellow screw cap *(provided with GRADE semi-annual collection kit)*

**Note: the lab ID label on one cryovial (for serum) will be different from the lab ID label on the second cryovial (for urine). Use extra care when aliquoting EDS visit samples to ensure that one participant's samples are aliquoted appropriately.

2.5 When the patient arrives for the visit, record the Participant ID number and any other identifiers on the EDS Specimen Transmittal Form. Also, enter the collection date and visit number. For the EDS baseline and annual visits, affix one of the 'EXTRA' lab ID labels from the baseline or annual visit lab ID label sheaf, as appropriate. For the EDS semi-annual visits, affix one of the 'EXTRA' lab ID labels from the label sheaf provided with the 'EDS or VB12' semi-annual collection kit.

3. Blood Collection

For detailed venipuncture instructions, see Section 5.2 beginning on page 10.

3.1 Perform the venipuncture filling each blood tube as completely as possible (i.e., until the vacuum is exhausted and blood flow ceases).

3.2 After the 2.5mL SST tube is filled with blood, gently invert 8 times. Place upright in a room temperature rack. Allow blood to clot at room temperature for at least 30 minutes but not longer than 45 minutes prior to centrifugation. Set a timer.

3.3 Transport all tubes to the laboratory immediately for processing. Process these specimens promptly as directed so that the serum sample is frozen in a timely manner.

4. Processing

4.1 Remove 2.5-mL red stopper SST tube from the room temperature rack and place into a centrifuge trunnion. Balance the centrifuge, then centrifuge at 2,000 x g for 15 minutes.

4.1.1 Post-centrifugation: Match the Lab ID label that appears on the SST tube with that on the 2-mL cryovial. Using the plastic transfer pipette included in the kit, transfer at least 0.5 mL of serum into the cryovial. Fasten a red screw cap tightly onto the cryovial.

4.2 Place the aliquot rack containing the serum cryovial into a -70°C freezer (or see section 7.3 on page 20 for clinical centers without access to a -70°C freezer). The aliquot should freeze upright so the specimen does not freeze in the cap. Once frozen (approximately 60 minutes), transfer the serum cryovial to the 6" x 9" biohazard bag that also contains the participant's urine cryovial from the GRADE semi-annual visit.

4.3 Place one absorbent pad into the biohazard bag. This serves to soak up any leaks and spills that may occur during shipping.

4.4 Place the bag containing the cryovials in a -70°C freezer (or -20°C freezer if no access to -70°C freezer) until shipment to the CBL.

5. Shipment

5.1 Ship the frozen samples on dry ice to the CBL the day of collection.

5.1.1 Since EDS baseline and annual visit collections occur as part of the regular GRADE visit, no separate shipping is required.

5.1.2 The serum aliquot prepared for the semi-annual EDS visit is shipped with the frozen urine aliquot collected as part of the regular GRADE semi-annual visit.

5.1.3 Follow packaging and shipping instructions for the regular GRADE semi-annual visit in Sections 8 and 9 on pages 20-24.

6. Special instructions for EDS samples at Final run-in

6.1 Only in certain situations will sample be collected at the final run-in visit. No additional sample collection is required; however an additional serum aliquot will be prepared from the serum tube already collected.

6.1.1 Obtain a 2mL vial and red screw cap from extra supplies. Affix an extra lab ID label from the final run-in label set to the 2mL vial.

6.1.2 Affix an extra lab ID label from the final run-in label set to the EDSSTF.

6.1.3 After collection and centrifugation of the 2.5mL SST tube collected as part of the final run-in visit, prepare the 0.5mL aliquot normally made for testing of creatinine/eGFR at final run-in. Prepare a second 0.5mL aliquot into the tube prepared in step 2. Tightly fasten with red screw cap.

6.2 Ship all samples collected at final run-in (3mL whole blood tube, 2 serum aliquots) at refrigerated temperature to the CBL on the day of collection.

6.3 Follow packaging and shipping instructions for the regular GRADE final run-in visit in Sections 8 and 9 on pages 20-24.

7. Recollection of EDS samples

7.1 If an EDS sample must be recollected, another EDSSTF must be completed. Contact the coordinating center for detailed information on how to enter the second EDSSTF in MIDAS.

7.2 If fasting serum is being recollected at a baseline and the collection is <u>not</u> specifically for EDS:

7.2.1 First confirm that the EDS questionnaires will be completed at the time of blood collection; it is important that these be completed at the same time.

7.2.2 Collect, process and ship samples as described for a baseline visit collection. Note that there is <u>NO separate serum collection for EDS</u> at a baseline visit.

7.2.3 Complete the BASESTF to document collection of the baseline samples. Also complete EDSSTF to document the visit as an EDS visit so that appropriate testing can be done at the CBL.

7.3 If fasting serum is being recollected at an annual visit and the collection is not specifically for EDS:

7.3.1 Collect, process and ship samples as described for an annual visit collection. Note that there is NO separate serum collection for EDS at an annual visit.

7.3.2 Complete the ANNSTF to document collection of the samples. Also complete EDSSTF to document the visit as an EDS visit so that appropriate testing can be done at the CBL.

7.4 If serum is being recollected at a semi-annual visit:

7.4.1 Use an EDS semi-annual collection kit to collect, process and ship the serum sample, following the procedures described above in this appendix (Appendix K). Complete the EDSSTF to document recollection of the EDS serum.

7.4.2 If other semi-annual visit samples also require recollection, refer to the appropriate section of this MOP for their collection, processing and shipment. Complete the REDRAW STF as needed.

Semi-Annual Lab Visit including Emotional Distress Substudy (EDS) collection

Test	Tube Name	Fasting Condition	Collection kit used	Collection Tube	Visual Reference	Aliquot Vials/Caps	Collection/Processing Instruction
hsCRP	Tube #1	Non-Fasting Acceptable	**'EDS or VB12'** semi-annual kit	2.5 mL red-top with gel separator	Label tube 	Label vial 	• Fill **labeled** tube completely with blood • Invert tube 8 times • Allow tube to sit upright 30-45 min at Room Temperature to clot • Centrifuge tube at 2,000 x g for 15 min • Aliquot 0.5 mL serum into **1 labeled** 2-mL cryovial • Tightly fasten with red screw cap • Freeze upright at -70°C until shipment preparation
HbA1c	Tube #2	Non-Fasting Acceptable	Semi-annual	3 mL purple-top EDTA	Label tube 	Do Not Aliquot	• Fill **labeled** tube completely with blood • Invert tube 8 times • **Do not centrifuge this tube!** • **Refrigerate** tube until shipment preparation
Urine Alb:Creat	Urine Cup	Non-Fasting Acceptable	Semi-annual	Urine Cup	Label cup 	Label vial 	• Mix urine cup completely • Aliquot 2 mL of urine into **1 labeled** 2-mL cryovial • Tightly fasten yellow screw cap on cryovial • Freeze upright at -70°C until shipment preparation

Appendix L. Guidelines for Collection of Biospecimens When a Participant is Not Fasting

This appendix describes the collection of biospecimens at an annual visit when the participant is not fasting. Participants are normally instructed to refrain from taking metformin and their diabetes medications before the annual visit; this is for safety reasons. If the participant does take either their metformin or diabetes medications, they are still considered to be fasting; however, they are not eligible for the OGTT, if the annual visit includes that assessment. This appendix also describes how to complete the collection of the sample set for the visit when the participant returns for the next visit (if the participant is willing to complete the collection).

At the annual visit, when the participant is **not** fasting:

- Collect 2.5mL SST, process to 1 cryovial (aliquot 1)
  - Label this aliquot with one of the 'extra' labels and write 'serum' on the label. Fasten a red cap to the vial.
  - Serum creatinine will be tested from this aliquot and eGFR calculated.
- Collect 3mL EDTA tube, no processing
  - HbA1c will be tested from this tube.
- Collect urine cup, process to 9 cryovials (aliquots 15 – 23)
  - Urine creatinine and albumin will be tested and ACR calculated from aliquot 15.
- Complete the ANNSTF, marking collected vials collected and shipped as appropriate.
  - Answer question 7 regarding fasting and OGTT eligibility status as needed.

Then, when the participant comes back and **is fasting**, the annual visit collection can be completed; if possible, use the remaining supplies from the annual collection kit (and same lab ID number labels) as was used for the non-fasting collection:

- Collect 9mL SST, process to 7 cryovials (aliquots 1 - 7)
  - Lipids will be tested from aliquot 1.
- Collect 6mL EDTA tube, process to 4 cryovials (aliquots 8 - 11)
- Collect 3mL EDTA tube, add aprotinin, process to 3 cryovials (aliquots 12 – 14)
- If an OGTT is part of the annual visit assessment, collect all timepoints and process for aliquots 24-46.
- Refer to Appendix J for details of sample documentation on the REDRAW STF and integration of the collection into another scheduled visit or an unscheduled visit.

Appendix M. Laboratory Alert Values

The CBL will send an alert email to notify clinical centers when the following laboratory values are determined for a GRADE participant.

Test	Notification/alert value	Applies to visits:
HbA1c	7.0% and higher	Quarterly; Confirmation (Interim*); Semi-annual; Annual
LDL, calculated	≥ 160 mg/dL	Baseline; Annual
Triglycerides	≥ 500 mg/dL	Baseline; Annual
Albumin/creatinine ratio (ACR)	> 300.00 mg albumin/g creatinine	Baseline; Semi-annual; Annual; Interim
eGFR**	< 30 ml/min/1.73m^2	Screening***; Final run-in; Annual; Interim
eGFR**	30 - 45 ml/min/1.73m^2	Final run-in; Annual; Interim
Vitamin B12	<300 pg/mL	Semi-annual; Annual

*Interim visits are those visits conducted according to the study MOP and can include visits to confirm an A1c outcome or to monitor eGFR. Interim ACR visits are not a GRADE study MOP defined visit, but have been used on an *ad hoc* basis at clinic/PI request.
**These alerts will be effective with release of Protocol version 1.6.
***The CBL does not perform screening visit testing, so no eGFR alerts are sent by email for these visits.

Appendix N. Collection of Microbiome Repository Samples

Stool samples will be collected prior to the baseline visit and around the first (6 month) semi-annual visit. These collections will take place in the participants' homes and the samples will be mailed directly to the CBL. The stool samples will form a microbiome repository.

An initial shipment of kits will be automatically mailed to each clinical center; the number of kits to be mailed will be determined by the Coordinating Center. After the initial shipment of kits, the clinical sites are responsible for requesting additional kits via the online supply re-order form. A very limited number of extra kits will be held at the CBL. Clinical sites should not request kits solely to have extra 'on hand.' One additional kit will also be provided to each clinical center to use as a demo kit to explain the collection procedures to the participant.

Clinical site coordinator responsibilities:

1. Maintain inventory of kits.
2. Request additional kits from the CBL after the initial automatic shipment of kits.
3. Prepare kit before providing to participant.
   a. Remove 1 of the barcode labels from the outside of the kit pouch and affix to MBIOSSTF. This is the lab ID label and number for this collection; it is a 14-digit number as opposed to the 8-digit lab ID number labels included with the blood collection kits. Note: you may need to use scotch tape to secure the lab ID label to the STF.
   b. Print and complete MBIOSSTF: site number, participant ID, GCODE and visit number. Other areas are left blank for the participant to complete. Make a copy for your records.
   c. Write participant ID number and site number on the back of the padded mailing envelope (the one pre-addressed to the CBL). This information will be used if the participant forgets to include the MBIOSSTF with the sample.
4. Provide instruction to participant for use of kit.
   a. Emphasize importance that only a pea-size amount of stool sample is needed in the collection tube. The tube does **not** need to be and should **not** be filled.
   b. Emphasize that collection tube is mailed in padded envelope to the CBL; questionnaires are mailed back to your clinic (see #4, below).
5. Provide participant with collection kit at final run-in visit and prior to the 06 month visit
   a. A postage-paid, pre-addressed envelope should be prepared and provided along with the collection kit for return of the questionnaires to the clinical center. Your site is responsible for providing the postage and envelopes.

CBL responsibilities:

In addition to processing and maintaining storage of the returned stool samples, the CBL will:

1. Provide a pre-determined number of kits to each clinic as part of an initial shipment
2. Inform the clinical site of sample receipt. This will be done via a lab result report sent the day after sample receipt.
   a. If the collection was not successful and no baseline visit sample can be stored, the site will be notified so they may plan for no 6-month collection from that participant.
   b. The CBL will also send a copy of the MBIOSSTF by email to the appropriate clinical center for completion of MIDAS entry. This copy will be sent no less frequently than monthly.

Return of unused kits:

Clinical centers may retain any kits that are not distributed for baseline collections. Provided the tube is not expired, the kit may be used for distribution for the semi-annual 06 month collection. The CBL may contact you to provide information on the number of kits in your inventory so that the appropriate number of kits for the semi-annual visits can be provided.

A participant who was provided with a microbiome kit at the final run-in visit, but was not eligible for randomization may return the unused kit to the CBL. The participant should return the kit by enclosing the unopened collection kit pouch, cardboard divider, paper toilet accessory and adhesive seal biohazard bag in the padded envelope pre-addressed to the CBL. The gloves and instruction sheet may be discarded.

Microbiome kit lot number TG57

The manufacturer of the microbiome collection kits has extended the expiration date of the tubes labeled as lot number TG57. The expiration date printed on these tubes is 7/22/2017. The manufacturer has extended the expiration date to 1/22/2018. Please continue using lot number TG57 collection kits until 1/22/2018.

Appendix O. Guidelines for Collection of Biospecimens for Vitamin B12 Measurement

This appendix describes the collection of biospecimens for vitamin B12 (VB12) measurement. Participants will have at least two VB12 measurements during the study: the first at their next semi-annual or annual GRADE visit (following initial implementation of the VB12 testing), and the second measurement will be performed as part of the 48 month annual visit. An additional VB12 collection and test will be performed at the next semi-annual or annual visit if the VB12 concentration is < 300pg/mL at either of the scheduled collections. If the VB12 biospecimen will be collected at the annual visit, no additional collection kits or tubes will be required. If the VB12 biospecimen will be collected at the semi-annual visit, an additional biospecimen will be collected using a separate collection kit. Please refer to the table below for a summary of VB12 biospecimen collection by visit.

Summary table of vitamin B12 biospecimen collection by visit

VB12 visit	Additional collection required?	Collection kit(s) used	STFs completed	Test to perform (at CBL)
Semi-annual	YES	Semi-annual; 'EDS or VB12' semi-annual	QTSMISTF; B12STF	Vitamin B12
Annual	NO	Annual	ANNSTF; B12STF	Vitamin B12

1. Preparation

1.1. The participant will be fasting for the annual visit collection, but fasting is not required at the semi-annual visit.

1.2. The CBL will provide a separate collection kit for collection of a serum tube at the semi-annual visit. This kit is named the ''EDS or VB12' semi-annual' collection kit and can be requested via the online supply re-order website. No separate collection kit is required at the annual visits.

1.2.1 The 'EDS or VB12' semi-annual kit may be used in either of 3 situations:

- A semi-annual visit where only an EDS collection will take place. See Appendix K for more information about collections for EDS measurements.
- A semi-annual visit where only a vitamin B12 collection will take place.
- A semi-annual visit where both an EDS and vitamin B12 collection will take place.

1.2.2 Do not worry if there are concerns that the EDS testing will take place when only the vitamin B12 testing is desired, or vice versa. **As long as the correct STF accompanies the sample the correct testing will occur.**

1.3. The B12STF should be completed for all vitamin B12 visits, regardless of whether or not a separate collection is done.

2. Organization

2.1. Prior to a patient's semi-annual vitamin B12 visit, remove the sheaf of barcoded Lab ID labels and the blood collection tube from the 'EDS or VB12' collection kit.

2.2. Affix one 'EDS or VB12' kit Lab ID label to the SST blood collection tube. Once blood processing is complete and the B12 specimen transmittal form has been completed, discard any remaining Lab ID labels from the 'EDS or VB12' kit.

2.3. Arrange the set of blood collection tubes in a rack in *the order in which they will be collected, as follows:*

Vitamin B12 SEMI-ANNUAL VISIT

Tube #1 red stopper SST tube *(provided with 'EDS or VB12' collection kit)*

Tube #2 3-mL purple stopper EDTA tube *(provided with GRADE semi-annual collection kit)*

One urine container *(provided with GRADE semi-annual collection kit)*

**Note: the lab ID label on tube #1 (SST) will be different from the lab ID labels on tube #2 (EDTA) and the urine container. Use extra care when collecting vitamin B12 samples to ensure that the participant's samples are collected and labeled appropriately.

2.4 Prepare a tray of the cryovials and color-coded screw caps that will contain the final samples to be shipped to the CBL. **The Lab ID labels on these vials must match those affixed to the corresponding set of blood collection tubes.**

Affix Lab ID labels to all of the cryovials in the kit prior to blood collection.

Vitamin B12 SEMI-ANNUAL VISIT

a. One 2-mL cryovial with red screw cap *(provided with 'EDS or VB12' collection kit)*
b. One 2-mL cryovial with yellow screw cap *(provided with GRADE semi-annual collection kit)*

**Note: the lab ID label on one cryovial (for serum) will be different from the lab ID label on the second cryovial (for urine). Use extra care when aliquoting vitamin B12 samples to ensure that one participant's samples are aliquoted appropriately.

2.5 When the patient arrives for the visit, record the Participant ID number and any other identifiers on the B12 Specimen Transmittal Form. Also, enter the collection date and visit number. For the B12 annual visits, affix one of the 'EXTRA' lab ID labels from the annual visit lab ID label sheaf. For the vitamin B12 semi-annual visits, affix one of the 'EXTRA' lab ID labels from the label sheaf provided with the 'EDS or VB12' semi-annual collection kit.

3. Blood Collection

For detailed venipuncture instructions, see Section 5.2 beginning on page 10.

1.1 Perform the venipuncture filling each blood tube as completely as possible (i.e., until the vacuum is exhausted and blood flow ceases).
1.2 After the 2.5mL SST tube is filled with blood, gently invert 8 times. Place upright in a room temperature rack. Allow blood to clot at room temperature for at least 30 minutes but not longer than 45 minutes prior to centrifugation. Set a timer.
1.3 Transport all tubes to the laboratory immediately for processing. Process these specimens promptly as directed so that the serum sample is frozen in a timely manner.

4. Processing

4.1 Remove 2.5-mL red stopper SST tube from the room temperature rack and place into a centrifuge trunnion. Balance the centrifuge, then centrifuge at 2,000 x g for 15 minutes.
4.1.1 Post-centrifugation: Match the Lab ID label that appears on the SST tube with that on the 2-mL cryovial. Using the plastic transfer pipette included in the kit, transfer at least 0.5 mL of serum into the cryovial. Fasten a red screw cap tightly onto the cryovial.
4.2 Place the aliquot rack containing the serum cryovial into a -70°C freezer (or see section 7.3 on page 20 for clinical centers without access to a -70°C freezer). The aliquot should freeze upright so the specimen does not freeze in the cap. Once frozen (approximately 60 minutes), transfer the serum cryovial to the 6" x 9" biohazard bag that also contains the participant's urine cryovial from the GRADE semi-annual visit.
4.3 Place one absorbent pad into the biohazard bag. This serves to soak up any leaks and spills that may occur during shipping.

4.4 Place the bag containing the cryovials in a -70°C freezer (or -20°C freezer if no access to -70°C freezer) until shipment to the CBL.

5. Shipment

5.1 Ship the frozen samples on dry ice to the CBL the day of collection.
5.1.1 Since vitamin B12 annual visit collections occur as part of the regular GRADE visit, no separate shipping is required.
5.1.2 The serum aliquot prepared for the semi-annual vitamin B12 visit is shipped with the frozen urine aliquot collected as part of the regular GRADE semi-annual visit.
5.1.3 Follow packaging and shipping instructions for the regular GRADE semi-annual visit in Sections 8 and 9 on pages 20-24.

6. Repeat testing of vitamin B12

6.1 If a vitamin B12 concentration < 300pg/mL is determined by the CBL, testing must be repeated in 6 months.
6.1.1 If the repeat testing is being done at a semi-annual visit, use an 'EDS or VB12' semi-annual collection kit to collect, process and ship the serum sample, following the procedures described above in this appendix (Appendix O). If done at an annual visit, then no additional collection is needed. In either case, complete the B12STF to document collection of the serum for vitamin B12; record the visit number as the visit at which the collection actually takes place.

7. Recollection of vitamin B12 samples

7.1 If a vitamin B12 sample must be recollected for purposes other than repeat testing as described above in section 6, another B12STF must be completed. Contact the coordinating center for detailed information on how to enter the second B12STF in MIDAS.
7.2 If fasting serum is being recollected at an annual visit and the collection is not specifically for vitamin B12:
7.2.1 Collect, process and ship samples as described for an annual visit collection. Note that there is NO separate serum collection for vitamin B12 at an annual visit.
7.2.2 Complete the ANNSTF to document collection of the samples. Also complete the B12STF to document the visit as a vitamin B12 visit so that appropriate testing can be done at the CBL.
7.3 If serum is being recollected at a semi-annual visit:
7.3.1 Use an 'EDS or VB12' semi-annual collection kit to collect, process and ship the serum sample, following the procedures described above in this appendix (Appendix O). Complete the B12STF to document recollection of the serum for vitamin B12.
7.3.2 If other semi-annual visit samples also require recollection, refer to the appropriate section of this MOP for their collection, processing and shipment. Complete the REDRAW STF as needed.

Semi-Annual Lab Visit including Vitamin B12 sample collection

Test	Tube Name	Fasting Condition	Collection kit used	Collection Tube	Visual Reference	Aliquot Vials/Caps	Collection/Processing Instruction
Vitamin B12	Tube #1	Non-Fasting Acceptable	**'EDS or VB12'** semi-annual kit	2.5 mL red-top with gel separator	Label tube 	Label vial 	• Fill **labeled** tube completely with blood • Invert tube 8 times • Allow tube to sit upright 30-45 min at Room Temperature to clot • Centrifuge tube at 2,000 x g for 15 min • Aliquot 0.5 mL serum into **1 labeled** 2-mL cryovial • Tightly fasten with red screw cap • Freeze upright at -70°C until shipment preparation
HbA1c	Tube #2	Non-Fasting Acceptable	Semi-annual	3 mL purple-top EDTA	Label tube 	Do Not Aliquot	• Fill **labeled** tube completely with blood • Invert tube 8 times • **Do not centrifuge this tube!** • **Refrigerate** tube until shipment preparation
Urine Alb:Creat	Urine Cup	Non-Fasting Acceptable	Semi-annual	Urine Cup	Label cup 	Label vial 	• Mix urine cup completely • Aliquot 2 mL of urine into **1 labeled** 2-mL cryovial • Tightly fasten yellow screw cap on cryovial • Freeze upright at -70°C until shipment preparation

Appendix P. Guidelines for Collection of Biospecimens for the CGM Study

This appendix describes the collection of biospecimens for the CGM study. A CGM visit will occur approximately 2 – 4 weeks prior to a participant's quarterly, semi-annual or annual visit (the 'CG' visit). At this visit, blood will be collected for measurement of glycated albumin and HbA1c. At a quarterly or semi-annual follow-up visit, additional blood will be collected for measurement of glycated albumin, along with the regularly-scheduled blood collection. No additional sample collection is required for the CGM study conducted at the annual follow-up visit. ***It is critical that the whole blood from a quarterly, semi-annual or annual follow-up visit be shipped the day of collection.*** Please refer to the table below for a summary of CGM study collections by visit.

Summary table of CGM study by visit

Visit	Additional collection required?	Collection kit used	STFs completed	Tests to perform (at CBL)
CGM ('CG')	YES	CGM	CGMSTF	HbA1c, glycated albumin
CGM follow-up visit (15, 18, 21)	YES	CGM follow-up kit	QTSMISTF; CGMSTF	HbA1c (as part of quarterly or SA visit), glycated albumin, red cell volume
CGM follow-up visit (12, 36, 60)	NO	Annual	ANNSTF; CGMSTF	HbA1c (as part of annual visit), glycated albumin, red cell volume

1. Preparation

1.1. The participant will be fasting for the annual follow-up visit collection, but fasting is not required at the CGM visit, nor the CGM follow-up visits that occur at 15, 18 and 21 months.

1.2. The CBL will provide a kit for collection of an EDTA tube and a serum tube at the CGM visit. This kit is named the ''CGM collection kit" and can be requested via the online supply re-order website. The CBL will also provide a kit for collection of a serum tube at the follow-up visits that occur at 15, 18 and 21 months; this kit is named the "CGM follow-up collection kit". No separate collection kit is required for the annual visits.

1.3. The CGMSTF should be completed for all CGM study visits, regardless of whether or not a separate collection is done (i.e at the CGM and all follow-up visits).

2. Organization

2.1. Prior to a patient's CGM or follow-up (15, 18, 21 months only) visit, remove the sheaf of barcoded Lab ID labels and the blood collection tubes from either the CGM collection kit or the CGM follow-up kit.

2.2. Affix one CGM kit Lab ID label to the SST blood collection tube and one CGM kit Lab ID label to the EDTA blood collection tube. For follow-up visits, affix one kit Lab ID label to the SST blood collection tube. Once blood processing is complete and the CGM specimen transmittal form has been completed, discard any remaining Lab ID labels from either kit.

2.3. Arrange the set of blood collection tubes in a rack in *the order in which they will be collected, as follows:*

CGM VISIT
Tube #1 red stopper SST tube
Tube #2 3-mL purple stopper EDTA tube

CGM FOLLOW-UP VISIT (15, 18 and 21 months)
Tube #1 red stopper SST tube

2.4 Prepare a tray of the cryovial and color-coded screw cap that will contain the final sample to be shipped to the CBL. **The Lab ID label on this vial must match that affixed to the corresponding set of blood collection tubes.**

Affix Lab ID label to the cryovial in the kit prior to blood collection.

CGM VISIT
One 2-mL cryovial with red screw cap

CGM FOLLOW-UP VISIT (15, 18 and 21 months)
One 2-mL cryovial with red screw cap

2.5 When the patient arrives for the visit, record the Participant ID number and other participant identifiers on the CGM Specimen Transmittal Form. Also enter the collection date/time; for the CGM visit, enter visit code 'CG'; for the follow-up visits, enter the visit code number (12, 15, 18, 21, 36 or 60). For the CGM visit or the 15, 18 and 21 month follow-up visit, affix one of the 'EXTRA' lab ID labels from the label sheaf provided with the collection kit (6xxxxxxx label series). For the CGM annual visits, affix one of the 'EXTRA' lab ID labels from the annual visit lab ID label sheaf (7xxxxxxx labels series) to the STF.

3. Blood Collection

For detailed venipuncture instructions, see Section 5.2 beginning on page 10.

3.1 Perform the venipuncture filling each blood tube as completely as possible (i.e., until the vacuum is exhausted and blood flow ceases).
3.2 After the 2.5mL SST tube is filled with blood, gently invert 8 times. Place tube upright in a room temperature rack. Allow blood to clot at room temperature for at least 30 minutes but not longer than 45 minutes prior to centrifugation. Set a timer.
3.3 After the 3mL EDTA tube is filled with blood, gently invert 8 times. Place tube upright in a room temperature rack, then place tube in the refrigerator (2-8°C) until shipment to the CBL. Refrigerate the tube in an upright position, if possible. **Do not centrifuge this tube!**
3.4 Transport all tubes to the laboratory immediately for processing. Process these specimens promptly as directed.

4. Processing

4.1 Remove 2.5-mL SST tube from the room temperature rack and place into a centrifuge trunnion. Balance the centrifuge, then centrifuge at 2,000 x g for 15 minutes.
4.2 Post-centrifugation: Match the Lab ID label that appears on the SST tube with that on the 2-mL cryovial. Using the plastic transfer pipette included in the kit, transfer at least 0.5 mL of serum into the cryovial. Fasten a red screw cap tightly onto the cryovial.
4.3 Store prepared serum cryovial in refrigerator (2-8°C) until shipment to the CBL.

5. Packaging and Shipment

5.1 For the CGM visit, wrap the serum cryovial and EDTA whole blood tube in a paper towel, then place the wrapped tubes in a 6" x 9" biohazard bag. For the 15, 18 and 21 month follow-up visit, wrap the serum cryovial in a paper towel, then place the wrapped vial in a 6" x 9" biohazard bag. Package only 1 participant's samples per bag. Place one absorbent pad into the biohazard bag. This serves to soak up any leaks and spills that may occur during shipping.

5.1.1 Ship the refrigerated temperature samples from the CGM visits and the 15, 18 and 21 month follow-up visits to the CBL the day of collection. Follow shipping instructions as for a GRADE quarterly visit, described in Sections 8 and 9 on pages 20-24.

5.1.2 Since CGM follow-up visits at 12, 36 and 60 months occur as part of the regular GRADE visit, no separate shipping is required. Follow annual visit shipping procedures described in Sections 8 and 9.

5.2 For follow-up visits conducted at 12, 15, 18, 21, 36 or 60 months the whole blood sample MUST be shipped the same day as it is collected. It is important that the CBL receives the sample within ~24 hours of collection to ensure stability of sample.

5.2.1 If one of these visits is scheduled for a Saturday, please contact the CBL to discuss a shipping option using the Nanocool temperature assurance shippers that will provide an extended cooling time. However, these shippers are expensive so Saturday visits should be avoided if possible.

6. Shipment of CGM Sensors to CBL

6.1 Participants will return their CGM sensors to the clinic. The clinic will then be responsible for sending the sensors to the CBL for download of the data. Clinics will batch ship the sensors, sending one shipment every two weeks. Shipments may be sent on any day of the week, Monday – Friday.

6.2 Upon receipt of sensor from participant, complete a CGMSHIP form. Record your site number, participant ID, GCODE, annual visit number, sensor serial number and the date the sensor is received from participant. On the date the sensor is shipped to the CBL, record the shipment date on the form.

6.3 Place the sensor in a 6 x 9” biohazard bag and seal bag. Place this bag in a 6 x 10” bubble mailer and seal mailer. Write the participant ID on the outside of the mailer. **DO NOT** place the CGMSHIP form inside the mailer!

6.4 Place all mailers containing sensors in a FedEx UN3373 clinical pak. Up to 8 mailers can be placed in 1 clinical pak. Make a photocopy of all CGMSHIP forms; place the original CGMSHIP forms inside the clinical pak and keep the copy with your records. It is important that the CGMSHIP forms be placed in the clinical pak **separately** from the sensors. This will help to prevent a sensor from being overlooked.

6.5 Affix barcoded FedEx airbill labeled for ‘2 day’ shipping to the clinical pak containing the sensors. See Appendix Q for example of this airbill.

CGM Visit ('CG')

Test	Tube Name	Fasting Condition	Collection kit used	Collection Tube	Visual Reference	Aliquot Vials/Caps	Collection/Processing Instruction
Glycated albumin	Tube #1	Non-Fasting Acceptable	CGM	2.5 mL red-top with gel separator	Label tube 	Label vial 	• Fill **labeled** tube completely with blood • Invert tube 8 times • Allow tube to sit upright 30-45 min at Room Temperature to clot • Centrifuge tube at 2,000 x g for 15 min • Aliquot 0.5 mL serum into **1 labeled** 2-mL cryovial • Tightly fasten with red screw cap • **Refrigerate** cryovial until shipment preparation
HbA1c	Tube #2	Non-Fasting Acceptable	CGM	3 mL purple-top EDTA	Label tube 	Do Not Aliquot	• Fill **labeled** tube completely with blood • Invert tube 8 times • **Do not centrifuge this tube!** • **Refrigerate** tube until shipment preparation

CGM Follow-up Visit at 15, 18 or 21 months

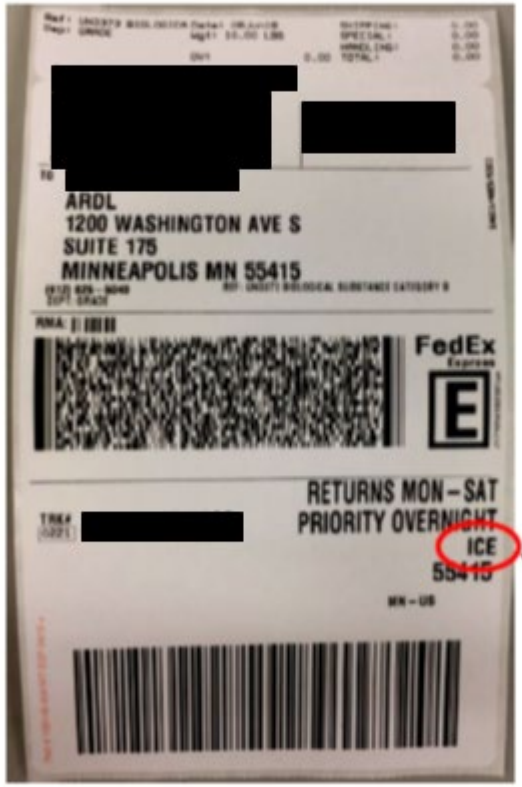
Test	Tube Name	Fasting Condition	Collection kit used	Collection Tube	Visual Reference	Aliquot Vials/Caps	Collection/Processing Instruction
Glycated albumin	Tube #1	Non-Fasting Acceptable	CGM follow-up	2.5 mL red-top with gel separator	Label tube 	Label vial 	• Fill **labeled** tube completely with blood • Invert tube 8 times • Allow tube to sit upright 30-45 min at Room Temperature to clot • Centrifuge tube at 2,000 x g for 15 min • Aliquot 0.5 mL serum into **1 labeled** 2-mL cryovial • Tightly fasten with red screw cap • **Refrigerate** cryovial until shipment preparation

Appendix Q. FedEx Pre-barcoded Airbill Usage

Use this label on **FedEx clinical paks** with refrigerated temperature specimens

blank

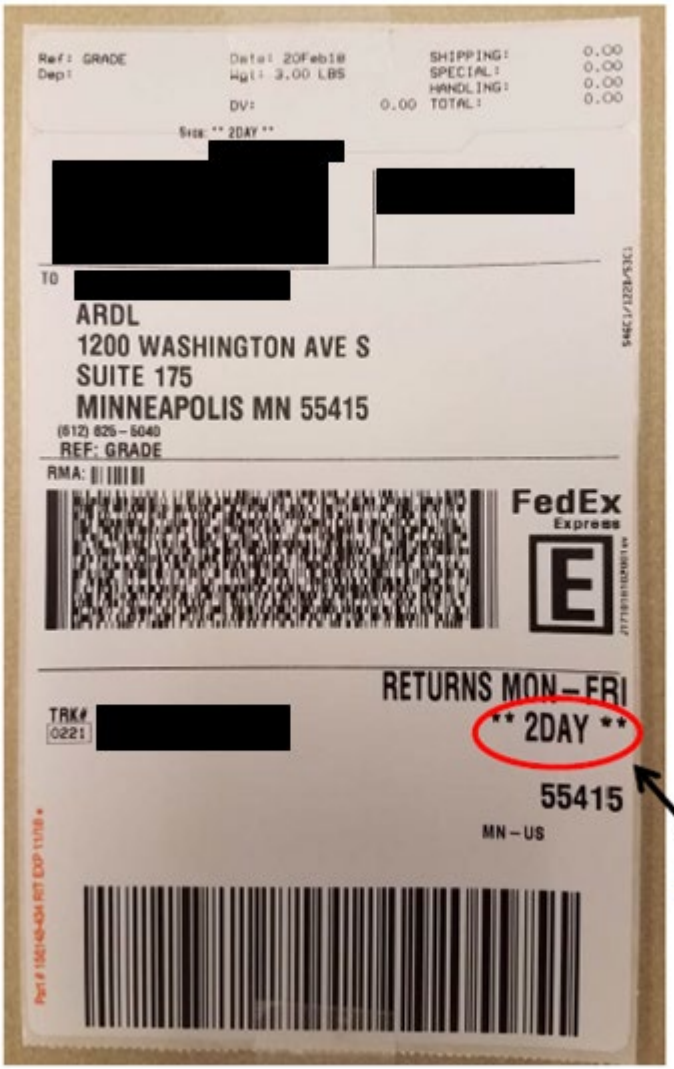
Use this label on shipments with **dry ice**



says 'ice'

Appendix Q. FedEx Pre-barcoded Airbill Usage

Use this label on CGM sensor shipments



Says '2DAY'