

## PREMIER Clinical Manual of Procedures

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## **PREMIER Clinical Manual of Procedures**

### **Summary of Edits**

#### **Summary of changes between Version 1.0 and 1.1:**

- Added procedure for CDC labs: folate, carotenoids, vitamin B-12.

#### **Summary of changes between Version 1.1 and 1.2:**

- Minimum amount of serum needed for CDC samples is 1 ml per vial.

#### **Summary of changes between Version 1.2 and 1.3:**

- If procedures for 24-hour urine refrigeration are not followed, urine samples stored for more than two days at room temperature are unusable.
- Added reference to additional specimen transport procedures in Chapter 27. Offsite Data Collection Visits.
- Updated shipping instructions to meet predetermined assay date.
- Updated contact person information at CLCS and BBI.
- Updated alert values for glucose and LCL-C.
- 8 ml purple cap transport vial for homocysteine not needed at 18 months

#### **Summary of changes between Version 1.3 and 1.4:**

- Updated contact person information at CDC on p. 21- 4 and 21-20.
- Updated statement on p. 21- 4 that states CDC shipment should not be sent to address stated on p.21-4 but to address on p.21-20.
- Updated shipping instructions for CDC on p. 21-20 to include timeframes for shipping samples, Premier Study Tracking # and primary contact persons for shipping and specimen questions.
- Added inclusion of Premier Study Tracking # in shipping paperwork to CDC on p. 21-19
- Added minimum levels of dry ice in CDC shippers on p.21-19.

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- 21. Central Laboratory Procedures

### ***Introduction***

The Core Laboratory for Clinical Studies (CLCS) of Washington University Medical School will serve as the central laboratory for PREMIER. BBI (Biotech Research Laboratories) will serve as the repository for the PREMIER storage specimens. This manual contains the information needed to collect, process, and ship specimens to the laboratory for analysis and the repository for storage. Please review the manual before the beginning of the study and contact the laboratory or the repository if you have any questions or require additional information.

### ***Contacts at CLCS***

Customer Service (314) 362-3522

Laboratory Fax Number (314) 362-4782

Primary Contact: Clinical Studies Coordinator  
Judy Jones (314) 747-1127

Laboratory Director: Thomas Cole, Ph.D.

Laboratory Manager: Connie Ferguson, M.T. (ASCP)

Information Systems Supervisor: Dave Gibson, B.S.

Technical Supervisor: Mike Macke, M.T. (ASCP)

U.S. Mail Address: Shipping Address: (UPS & FedEx)

Core Laboratory for Clinical Studies  
Washington University School of Medicine  
660 S. Euclid Ave., Box 8046  
St. Louis, MO 63110

Core Laboratory for Clinical Studies  
Lipid Research  
4940 Parkview Place  
St. Louis, MO 63110

### ***Contacts at CDC***

Christine M Pfeiffer, Ph.D.  
Chief, NHANES Laboratory  
Centers for Disease Control & Prevention  
4770 Buford Hwy. NE, MS F-18  
Atlanta GA 30341-3724 USA

e-mail: CPfeiffer@cdc.gov  
Phone: 770-488-7926  
Fax: 770-488-4609

***Note: CDC specimens are not shipped to above address. See p. 21-20 for a complete address.***

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### ***Contacts at BBI***

Primary Contact: Laboratory Manager  
Carla Hansen (301) 208-8100 Fax: (301) 208-8829

Principal Investigator  
Dr. Mark Cosentino (301) 208-8100

Shipping address: (UPS & FedEx)

Carla Hansen  
BBI - Biotech Research Laboratories  
217 Perry Parkway  
Gaithersburg, MD 20877

### ***Study Supplies***

#### ***Supplies provided by CLCS***

- Blood collection tubes
- Labels and vials for specimen aliquots
- Shipping and storage boxes for specimens
- Urine collection hats and jugs
- Blood collection kits for screening, 6 months and 18 months
- Shipping containers and supplies
- Pre-addressed Federal Express airbills, airbill pouches, dry ice labels, and instructions
- Disposable transfer pipettes

The CLCS will distribute specimen collection kits directly to each center as needed via UPS. These shipments require about 3-5 days shipping time. **Therefore, orders should be made at least a week in advance of the date that they are needed.** Requests for supplies can be made by telephone or fax to the CLCS Clinical Studies Coordinator. If making a request via fax, use the Request for Additional Lab Supplies (Form #315).

The provided supplies must be used only for this study. If a large number of redraws or abnormal situations occur, inform the CLCS of the need for replacement supplies. Each center will be responsible for maintaining an adequate inventory of supplies.

The CLCS will also provide the collection kits for the storage specimens. The BBI will provide the shipping container and supplies. The CC will contact the BBI after randomization to inform the repository of the number of specimens to expect from each site so they can send the correct number of shipping containers.

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### *Supplies provided by CDC*

- (1) 7-mL Hemogard-closure SST Vacutainers with no anticoagulant (pre-screened to ensure absence of background lead contamination).
- bar-coded labels for specimen vials (“VITA” and “SFOL”) and paperwork
- 2.0-mL Nalge cryovials
- reusable Styrofoam insulated shippers and extra outside cardboard covers
- clear plastic packaging tape and dispenser
- white 9x9 grid cardboard storage boxes to place vials into for shipment
- large ziplock bags for enclosing the white storage boxes during shipment (to contain any leakage which might occur)

### *Supplies required at clinical sites*

- Standard clinical centrifuge (a refrigerated centrifuge is preferred, but a non-refrigerated centrifuge is acceptable). The CDC lab requires a centrifuge capable of 1500 X G (2400-2800 RPM), with swing-out rotors.
- Ultra-low temperature freezer (-70C or colder, i.e., ultra low REVCO or equivalent). All blood specimens must be stored at -70C. If necessary, urine specimens may be stored in a non-cycling -20C freezer, for up to 30 days before shipping. Contact the CLCS if you do not have access to a -70C freezer.
- Dry ice for shipping.
- Federal Express pick-up service, UPS drop-off service
- Racks for tubes
- Phlebotomy supplies
- Distilled or de-ionized water
- Indelible markers for labels
- Biological waste bags
- 6 N HCl preservative for urine
- 2 L graduated cylinder for measuring urine volume
- Graduated pipettes for aliquotting urine

### ***Preparing for the Visit:***

#### *Materials Needed*

Visit-specific kits (see description below)

Visit and participant specific labels

Central Lab Collection Form- 24-Hour Urine (Forms #20, 62, 64)

CDC Form (#77)

Central Lab Collection Form- Fasting Blood (Form #21, 63, 65)

Central Lab Freezer Log – Blood and Urine Samples (Form #328)

Storage Lab Freezer Log – Blood Samples (Form #329)

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Storage Lab Freezer Log – Urine Samples (Form #330)

Central Lab Duplicate Sample Form (Form #66) – if participant is part of the CLCS QC procedure

### *Description of Visit Kits used for CLCS Blood Draws*

A single kit of blood collection supplies will be used for all visits (screening, 6 months, and 18 months). Labels will be sent separately for each visit.

#### Supplies:

2 x 10 mL SST Vacutainer tubes (red/gray top)

1 x 13 mL plastic pooling vial (red cap)

1 x 8 mL plastic transport vial (red cap)

3 x 2 mL plastic freezing vials (red cap)

2 x 10 mL EDTA Vacutainer tubes (purple top)

1 x 13 mL plastic pooling vial (purple cap)

1 x 8 mL plastic transport vial (purple cap – not needed at 18 months)

3 x 2 mL plastic freezing vials (clear cap)

#### *Buffy Coat (screening only):*

1 x 2 mL plastic freezing vial (purple cap)

#### Additional materials from CDC:

1 x 7mL Hemogard-closure SST Vacutainers (pre-screened to ensure absence of background lead contamination). If SST Vacutainers are used, it is much easier to transfer the serum to the vials and contamination with RBCs is minimized. Ordinary red-top Vacutainers may be used, but greater care is required for serum transfer.

2.0-mL Nalge cryovials

### *Description of Visit Kits used for Urine Collection*

Urine collection hats and jugs will be provided in bulk for distribution at visits screening, 6 months, and 18 months. Labels will be sent separately for each visit.

#### Supplies

6 x 8 mL plastic freezing vial (yellow cap)

### ***Preparing the Tube and Vial Labels***

1. Labels are provided for all tubes and vials. Blank specimen identification labels are provided for urine and bloods at screening visits. For CLCS and BBI specimens, labels will be pre-printed with participant's identification number for 6 month and 18 month visits and will be

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sent to the clinical sites via UPS once all patients for that cohort are randomized. For CDC specimens, labels are provided by CDC.

2. At screening visits, for all CLCS and BBI labels: Record the subject's unique 10-digit identification code. Record the draw date of the visit or urine collection start date.
3. CDC Labels: You will be provided with a set of 6 preprinted labels per participant per visit. The ID number consists of a 4-digit prefix, followed by a one or two digit suffix. The prefix is participant specific; the suffix refers to the visit (5 = baseline, 8 = 6 month, 10 = 18 month). *The prefix does not match, nor should it be confused with, the participant's PREMIER ID number.* For eligible participants, one label should be placed on the 7ml hemogard SST vacutainer tube for processing. One label should be placed on each of the CDC cryovials. Record the participant's PREMIER ID on the label, as well as the draw date. The labels should be placed on the cryovial so that the bar-code label is horizontal, or "stair step", (i.e. when the vial is standing upright the bar-code lines will run horizontal (side-to-side)). The vials cannot be read if the barcode is placed on the vial in a different manner from what is noted above. Place one of the extra labels on the Form 77 in the box marked "Affix CDC label". **It is vital that the label placed on the participant's Form #77 match the label on their cryovials. Failure to do this will result in the specimen being discarded for analysis.** Note: If a label becomes unusable during processing, affix one of the extra labels (with the appropriate visit number) to the cryovial.
4. At each intervention visit, all information will be pre-printed except for the draw date, which is recorded at the time of the visit.
5. Affix the completed labels to the appropriate tubes found in the kit before draw. Position the labels on the freezing vials over the white patch allowing the volume markings to remain visible.

*Note: It is imperative that all tubes are labeled correctly and completely, and that the writing be legible.*

In addition to blood chemistries being measured at other external laboratories, serum levels of fat-soluble vitamins (vitamins A/E/carotenes/retinyl esters) and folate and vitamin B12 will be measured by the NHANES Laboratory at the Centers for Disease Control and Prevention. The procedures outlined below apply to specimen collection and processing for these specimens in the study clinics. It will be very important to assure that continuity of all techniques is maintained throughout the study duration, to minimize variability. This section provides all necessary information for correct collection, processing, and shipment of the study blood specimens for analysis, as well as a summary of procedures used by the NHANES Laboratory when the specimens are received at CDC.

Venipuncture blood samples will be collected by the clinic on each study participant. Subjects must have fasted 12 hours prior to blood collection, with only water allowed. Fasting is required for baseline samples. If the subject has not been fasting, the baseline blood draw must be rescheduled. At follow-up visits, if the participant is not fasting, the sample may be drawn. In this case, note on the form whether fasting time is sufficient. Processed serum samples will be

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sent to the Centers for Disease Control and Prevention (CDC) for analysis and storage of any residual specimens.

**Do not use the CDC-supplied materials for collecting or processing blood for any other study tests; they have been pre-screened for background contamination and it is imperative to keep them as clean as possible.**

### ***Preparing the 24-hour Urine Collection Labels***

Four types of labels will be provided to place on each urine collection jug:

1. Collection instruction labels
2. Specimen identification and start/stop labels
3. Estimated spills labels
4. Collection procedure verification labels

Place all four labels on each urine collection jug prior to handing out to patients. The information on these labels is critical for proper collection documentation. Verify that all labels are properly completed when the jug is returned. If the patient requires more than one jug for collection, only one set of labels is required.

### ***Preparing Forms 20, 21, and 77***

1. Use one copy of each form and its worksheet per subject per visit. Fill out the top of each form (ID and Visit) and the top of each worksheet (ID) prior to the visit.
2. The remainder of each form will be completed at the visit.

### ***Preparing the Storage/Shipping Supplies***

1. A -70C freezer must be available for storing samples after collection and processing. Specimens will be shipped by Federal Express to the CLCS and BBI three times during each cohort. The screening shipment will go out after randomization and the intervention shipments will go out at the end of the 6 month and 18 month periods. Contact the laboratory and the repository prior to shipping.
2. You will need to store the specimens in boxes in the freezer until you are ready to send a shipment to CLCS or to storage. Complete the Freezing Log forms (Forms #328, 329, 330) when collecting the sample in order to record which sample is going into the slots in the freezer boxes. If you use freezer racks, create a site-specific Freezer Log form to track the locations of specimens. These forms are for internal use only. They will not be entered into the data entry system. See directions below for shipping the specimens.

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### ***At the Visit:***

#### *General Instructions*

Treat all materials that have been in contact with blood or urine as potentially infectious. Dispose of these materials, including needles, by approved procedures for the individual site. Wear gloves to minimize the transmission of infection. Observe universal precautions when handling potentially infectious material.

#### *Collection of Blood Samples*

A fasting blood must be collected between SV2 and randomization, and at the 6 month and 18 month visit.

Subjects must have fasted 12 hours prior to blood collection, with only water allowed. If the subject has not been fasting, the visit must be rescheduled.

Due to the sensitivity involved in specific testing, standardization of specimen collection is imperative. The subject **MUST** be seated for at least 10 minutes prior to specimen collection. A tourniquet may be used for no longer than two minutes. Deviation from this standardized sample collection protocol will cause significant variability in assay results. Be consistent from visit to visit.

1. Clean with an alcohol pad, then draw blood from the crook of the arm, generally from the antecubital vein. Fully fill all tubes.
2. Draw the two 10-mL (red/gray top) SST serum tubes before the two 10-mL (purple top) EDTA plasma tubes. Non-additive tubes are drawn before additive tubes to avoid additive contamination of the non-additive tube. Cross-contamination between different additive tubes can also occur, making test results erroneous.
3. Thoroughly mix all tubes immediately after collection by gently inverting the tubes at least five times. *Do not shake.*
4. Remove the needle and apply pressure to the venipuncture site. Cover with an adhesive strip when the blood has stopped flowing.

The Central Lab Collection Form - Bloods (Form #21) is used for processing the fasting blood samples.

### ***Preparation and Blood Collection for Folate, Carotenoids and Vitamin B-12***

#### *Summary*

Collect and completely fill a 7-mL SST (“tiger-top”) Vacutainer [NO anticoagulant] from each study participant.

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### *Blood Collection*

Blood should be drawn from an antecubital vein or from some other convenient arm vein. Apply the tourniquet to locate the vein, then release it while the venipuncture site is being cleaned. Swab the venipuncture site with alcohol and allow it to dry. Reapply the tourniquet, reconfirm the vein location, and perform the venipuncture, using either a 21- to 23-gauge butterfly needle or a 21- to 23-gauge multi-sample Vacutainer needle. (Use of the butterfly needle will make tube changing easier since the needle can be taped in place.) Care should be taken to avoid protracted probing for vein location. Release the tourniquet before the needle is removed from vein. Prolonged application of the tourniquet should be avoided to minimize hemoconcentration.

Collect blood using a Vacutainer system following the instructions supplied with the tubes. (Training tapes are available from manufacturer, Becton-Dickinson, Rutherford, NJ.) The butterfly needles come complete with tubing and Luer adapters which fit into the Vacutainer holders, thus enabling the needle to be taped down to minimize movement while both hands may be used to change Vacutainers. Introduce the butterfly needle into the vein and fill the Vacutainers as completely as possible, collecting any other tubes with anticoagulant first, then the SST tube.

Use a dry gauze pad to apply pressure when removing the needle since a wet pad could result in fluid being drawn into the Vacutainer. After removing the needle, apply pressure firmly to the puncture site with the subject's arm held straight, rather than with the elbow bent, for at least 5 minutes to avoid hematoma formation. Re-examine the puncture site to verify that any residual bleeding has ceased, then apply a bandage as a precaution. Dispose of all needles and contaminated wastes properly in the biosafety container.

Make sure that each Vacutainer is clearly labeled with the subject's ID number, using the provided labels. Apply a study label for each page of the participant's paperwork as well.

### Offsite blood draws

Refer to procedures outlined in Chapter 27, Offsite Data Collection Visits, for additional information regarding transporting specimens from offsite locations.

### *Safety Note*

CDC recommends, as good laboratory practice that all blood specimens, used needles, etc., should be treated as though they were infectious for HIV and hepatitis B virus. All used needles and lancets should be placed in puncture-resistant containers; then, along with used gauze, Vacutainers, pipets, vials, Hemocue cuvettes, plastic-backed "diapers", etc., they should be autoclaved prior to final disposal. Use of disposable gloves when collecting and processing blood is also required. (See CDC recommendations for preventing transmission of infection with human HTLV III/LAV in the workplace. MMWR 37:377-388, 1988).

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### *Collection of Urine Sample*

Between SV2 and randomization, and at the 6-month and 18-month visit, instruct the participant to collect a 24-hour urine specimen (see appendix A for sample instructions for participants). 24-hour urine collection should not be done during menstruation. Schedule urine collection to avoid collections during this time.

In order to maximize quality control for urine collections, the collections should ideally begin on Monday through Thursday when the participants come in to pick up their collection materials, and participants should be instructed to return the collections the next day. This enhances the likelihood that the initial voiding to start the interval is discarded and that a final voiding is obtained at the end of the collection interval. It should also maximize the likelihood that the collection duration falls within the allowable limits (22-26 hours). Incentives might be useful to encourage collection during the week. If, in the opinion of clinic staff, the participant is unlikely to comply with the collection regimen due to this weekday collection, the participant may be allowed to collect the specimen over the weekend. In this case it is still preferable to either begin or end the collection in the clinic, so that at least some level of quality assurance is achieved.

1. Distribute the 24-hour container and instructions to the participant and review the instructions with the participant. The instructions are included in Appendix A below.
2. Make sure that all four information labels are affixed to the collection jug and that it is filled out with the appropriate identifying information. If the subject has a high urine output, two jugs may be required.
3. If the specimen is to be returned the next day, have the participant start the collection before leaving the clinic (i.e., void the bladder into the toilet). Inform the participant to bring the container back within 24 hours of collection. Specimens should be refrigerated or kept in a cool place during collection. The instructions for processing the specimen should be followed no matter when the specimen is returned.
4. Take the 24-hour urine container from the participant, check to make sure that the labels on the tab attached to the jug are filled out correctly and completely, and verify that the ID listed on the label matches that of the participant. Also confirm that the participant: voided her bladder at the start of collection and did not save the specimen, collected a final voiding at the end of the collection period, and returned the specimen within 24 hours of the final voiding.

The specimen is considered to be inadequate if any of the following are true.

- The total duration of the collection is less than 22 hours or greater than 26 hours
- The collection period did not start with an initial, discarded voiding
- More than one voiding (including the final voiding) was missed
- The total volume of the sample is less than 500 cc
- The urine is collected during menstruation

If the specimen is inadequate, or if the participant failed to bring it in, a second specimen must be obtained. Give the participant a new set of collection materials, attach and fill out the labels

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correctly. Save an aliquot from the original sample as a backup in case the participant is not able to provide an adequate sample, and note on the label that the sample was inadequate.

If the participant does not bring a repeat specimen, process the aliquot from the original (inadequate) sample in its stead, and note on the shipping label that the sample was inadequate and why. If both the samples are inadequate, send the better of the two samples.

Assuming that the participant does bring in a specimen, either immediately take it to the clinic's lab area for processing or place it in a refrigerator until it can be processed. Avoid leaving the specimen at room temperature for any longer than is necessary. In instances where these procedures weren't followed, specimens may be stored up to two days at room temperatures without affecting analyses. If stored for more than two days at room temperature, the specimen is unusable.

The Central Lab Collection Form - 24-hour urine (Form #20) is used for processing the 24-hour urine sample.

### ***After the Visit***

#### ***Process and Store Serum Specimens***

1. Allow the 2 x 10 mL SST tubes (red/gray top) to clot for 30-60 minutes at room temperature in an upright position. Verify that the specimen is fully clotted.
2. Centrifuge the clotted tubes for 15 minutes at 1,500 x g. After centrifugation, check the SST tubes for a complete gel barrier between the serum and the cells. Re-centrifuge if the barrier is incomplete or if red cells are seen above the barrier.
3. Pour the serum from the SST tubes into the 13 mL pooling vial. Cap the pooling vial and gently invert several times to obtain a homogeneous specimen.
4. Using a transfer pipette, transfer 1 mL of serum from the 13 mL pooling vial into 3 x 2 mL freezing vials with red caps, labeled "Storage Serum" and 3mL or the remaining serum into a 1 x 8 mL freezing vial with red cap labeled for "CLCS Serum." A minimum of 3 mL is required for analysis.
5. Fasten the appropriately colored caps tightly and immediately place the vials in freezer racks at -70°C. As the samples are put into the box or rack in the freezer, you can use the Central Lab Freezer Log – Blood and Urine Samples (#328), and the Storage Lab Freezer Log – Blood Samples (#330) to help keep track of specimen locations. Use the log to note the box or rack slot number, participant ID, visit, collection date, sample type (e.g., plasma, serum) and any comments about the particular sample. These forms are for internal use only. They will not be entered.
7. Fill out the worksheet for the Central Lab Collection Form- Fasting Blood (Form #21)
8. If a repeat draw is necessary, repeat the steps above.
9. After any repeat draws for this participant are complete, use the worksheet to complete Form #21

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### *Process and Store EDTA Plasma*

1. Centrifuge the 2 x 10 mL (purple top) EDTA tubes without delay at room temperature. Centrifuge at  $>1,500 \times g$  for 15 minutes to remove blood cells. No red cells should be present in the plasma or along the sides of the tubes.
2. Using a transfer pipette, transfer the plasma from both tubes into the 13 mL plastic pooling vial. Cap the pooling vial and invert several times to obtain a homogeneous specimen.
3. Using transfer pipettes, transfer 1 mL of the plasma from the pooling vial into 3 x 2 mL freezing vials (clear cap), labeled "Storage Plasma." Transfer the remaining plasma into the 1 x 8 mL freezing vial (purple cap), labeled "CLCS Plasma." A minimum of 1 mL is required for analysis.
4. At the screening visit only: Save the EDTA tubes that contain the cell pellet for the buffy coat. The buffy coat is the whitish layer of cells overlaying the packed red cells remaining in the EDTA tubes after the plasma is removed. Collect the buffy coats from both EDTA tubes and transfer into the 2 mL freezing vial with a purple cap labeled for "Buffy Coat."
5. Fasten the appropriately colored caps tightly and immediately place the vials in freezer racks at  $-70^{\circ}\text{C}$ . As the samples are put into the box or rack in the freezer, you can use the Central Lab Freezer Log – Blood and Urine Samples (#328), and the Storage Lab Freezer Log – Blood Samples (#330) to help keep track of specimen locations. Use the log to note the box or rack slot number, participant ID, visit, collection date, sample type (e.g., plasma, serum) and any comments about the particular sample. These forms are for internal use only. They will not be entered.
6. Fill out the worksheet for the Central Lab Collection Form- Fasting Blood (Form #21).
7. If a repeat draw is necessary, repeat the steps above.
8. After any repeat draws for this participant are complete, use the worksheet to complete Form #21.

### *Preparing Serum for Folate, Carotenoids and Vitamin B-12*

Fasting bloods are critical to an accurate evaluation of vitamins, particularly the fat soluble vitamins. Fasting for these blood draws is required at baseline. In follow-up every effort to collect fasting blood should be made. If fasting blood cannot be obtained, non-fasting blood should be collected and appropriately flagged on Form #77.

Avoid exposing these samples to light for any length of time. While it is not necessary to use amber tubes or to wrap the tubes in foil, do not process in front of a window. If transporting samples from a collection site to another location for processing or storage, enclose the tubes in a box or other light-protective container.

After the blood specimens have been drawn, allow the filled red-top or SST Vacutainers to remain at room temperature for 30-45 minutes **but no more than one hour** for complete blood coagulation. Centrifuge the SST tubes for 10-15 minutes at 2400-2800 RPM (1500 X G for most counter-top centrifuges, with swinging-bucket rotors). Do not open and "rim" tubes prior to centrifugation; this may introduce contamination. Be sure to use balance tubes in the centrifuge

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if necessary. The time required for centrifugation is a convenient time to prepare the remaining supplies for specimen processing, to label serum vials, and to create a specimen list.

Carefully open each centrifuged tube away from your face, following Universal precautions to minimize aerosol formation. Decant half of the serum into each of the CDC cryovials. A minimum of 1 ml of serum is needed for each vial. Be sure that each vial is correctly labeled with the study participant's ID and test name.

Place the filled cryovials for each subject in the white storage boxes to keep them upright during shipment. Start on the top left corner of the box (where the dot is) and place the samples as follows to have 4 sets of samples per row:

patient 1 vial 1, patient 1 vial 2, patient 2 vial 1, patient 2 vial 2, patient 3 vial 1,  
patient 3 vial 2, patient 4 vial 1, patient 4 vial 2, empty space.

Repeat this process with each row to the bottom of the box. Check off the spaces the transmittal sheets so that we will know that you successfully processed these vials in case of any discrepancies in shipment.

PREMIER Study samples will be collected at baseline, 6-month, and 18-month intervals. Since it may take several months for each clinic to collect their samples, the white storage boxes can be accumulated, stored at  $< -70$  EC, and shipped to CDC on a monthly basis until that interval's collection is complete. We will send at least 5 boxes to each clinic. (Each box holds 9 rows x 4 patient vial sets, or samples from 36 patients.)

### *Process and Store Urine*

1. Record the sample identification, dates and times on the Central Lab Collection Form- 24-hour Urine (Form #20).
2. Invert the sample container at least eight times to ensure a uniform sample.
3. Measure the total urine volume (use a graduated cylinder). Note the volume on Form #20.
4. Label and prepare 6 x 8 mL freezing vials (yellow cap) as follows:  
Tubes 1, 2, and 3: Add nothing to the vials labeled 'NO HCl'.  
Tubes 4, 5, and 6: Add 2 drops of 6 N HCl to the vials labeled 'With HCl'.
5. Add 5 mL of well-mixed urine to tubes 1-6, using a graduated pipette. Cap securely. Invert to mix.
6. Fasten the yellow caps tightly and immediately place the vials in freezer boxes or racks (in an upright position) at  $-70^{\circ}\text{C}$ . As the samples are put into the box or rack in the freezer, you can use the Central Lab Freezer Log – Blood and Urine Specimens (#328), and the Storage Lab Freezer Log – Urine Samples (#329) to help keep track of specimen locations. Use the log to note the box or rack slot number, participant ID, visit, collection date, sample type (e.g., plasma, serum) and any comments about the particular sample. These forms are for internal use only. They will not be entered.

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7. The remaining urine may be discarded. Be sure to use distilled/de-ionized water to rinse the graduated cylinders between samples to avoid cross-contaminating the specimen.
8. Fill out the worksheet for the Central Lab Collection Form- 24-hour Urine (Form #20).
9. If a repeat collection is necessary, repeat the steps above.
10. After any repeat collections for this participant are complete, use the worksheet to complete Form #20.

### *Complete Collection Forms*

Once specimen processing has been completed, transfer the data from the worksheets for the Central Lab Collection Forms (#20, #21, and #77) to the first page of each form. Send the forms to the data entry technician to be entered.

### *Lab QC*

To measure quality control and assay variability at the central lab, sites will be sending additional blinded specimens along with our regular shipments of participant specimens. The data entry system will print out reports for each participant for whom we will be sending a blinded specimen. Follow the instructions on the report to create the appropriate duplicate sample.

These duplicate samples are sent to the lab to verify the accuracy of their test results. Duplicate samples will be created in such a way that the central lab cannot readily identify them. This means that each sample has a fake ID, collection date, collection times, and other information. Duplicate specimens are obtained only during screening.

Form 66 will print out for randomly selected SV3 eligible participants when the SV3 visit form is entered (check printer). Once it has printed, check to see if the lab samples have already been collected. Most lab specimens are collected after SV3, but if this participant has already done their collection, just check "Yes" for the first question on the form. For forms where the first answer is "No," prepare for the duplicate collection by doing each of the items on the checklist. Refer to Form #66 coding instructions for more details.

Enter Form #66. Store the duplicate specimens in the same manner as the original specimens. When it comes time to ship lab specimens, the duplicates will show up on the packing and shipping logs with dummy ID numbers, just as if they were real participants. If the participant from whom the duplicate was collected is not randomized, you will be prompted to discard both their original and duplicate samples after the randomization period is over. (Central Lab Samples to Discard: LAB10 Report).

The coordinating center recognizes that creating the duplicates correctly is a difficult process. Please contact the Data Manager at the Coordinating Center if you have any questions or problems, or if there is any way the Coordinating Center can help smooth this process.

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### ***Shipping specimens to the central lab and storage lab***

#### *Data Entry/Data Management*

In order to be able to send the shipment to CLCS or to BBI, you must send the completed Forms 20 and 21 to the data entry technician to be entered. When you are ready to ship, print out the Central Lab Data Completeness Report (LAB05) from the PREMIER Lab Tracking System to see if there are any additional forms you need to enter. All Central Lab Collection forms for a particular visit should be entered before preparing boxes for shipment.

#### *Packing Boxes for Shipping*

1. The central lab and storage lab will provide 2" and 3" cardboard freezer storage boxes.
2. To simplify the process of shipping specimens, sort the specimens in freezer into samples going to CLCS (bloods and urines can go in same box) and samples going to BBI (bloods and urines in separate boxes). Within these three groups, sort by ID and collection date. Specimens can be stored in the shipping boxes, or in racks.
3. The PREMIER Lab Tracking System is used to prepare the samples for shipment. Be sure to read the users manual carefully before beginning the shipment process.
4. Open the PREMIER Lab Tracking Management System.
5. Create a Box: The first step in Lab Tracking is to create a box for CLCS or BBI. You will only be able to work on one box type at a time. The system will search for all your entered Central Lab Collection forms. It will come back and tell you if you do not have enough samples to fill a box. You can then choose to prepare that box (and ship it only partially full) or not. The system will then automatically print out a working draft of the selected shipping log and visual map for you to start packaging the specimens. The visual map is a map of the slots for the particular type of box you will use. It will fill the slots starting from the bottom left hand corner with the participants who have completed Central Lab Collection forms in the system. The shipping log will have the same information that is on the visual map plus space to write in the shipping condition.
6. Prepare a box: Use the working draft of the visual map to fill the slots of the shipping box. Due to the space required by caps, the 3" boxes will hold 36 instead of 49 vials. The fourth position in each row and the entire fourth row must be left empty for the vials to fit. The starting location for filling these boxes is the front, left corner of the box, moving to the right, then to the back as each row fills. Specimens are sorted within the box so each participant's specimens are grouped together. Specimens for a single participant will not be spanned across two boxes, so some slots in a box may not be filled.
7. Edit Specimen Shipment Conditions: If any specimens that have been lost, broken, lost labels or become unusable for some other reason, (1) record the condition of the specimen in the shipping condition column of the shipping log, (2) leave that slot in the box empty. If there are any other problems with the specimens (i.e., hemolyzed) and you still plan on shipping that specimen, record the problem with the specimen in the shipping condition column of the shipping log.

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8. Finalize a box: After all the specimens have been put in the box and the shipping conditions have been recorded. Note: the default shipping condition is “good”, you will only need to note any conditions that are not “good”. Go back into the Lab Tracking system and select a box to finalize. You will need to enter the following: Box preparer, prepared date, and any comments. Shipping conditions should have been edited prior to this step. Note: once you finalize a box, you will not be able to modify the data pertaining to that box.

### *Shipping Specimens*

1. Shipping will occur three times during a cohort. The first shipment will be sent to CLCS and BBI following randomization, a second shipment will be sent at the end of the 6-month visits, and a third shipment will be sent at the end of the 18-month visits. Contact the lab and the repository before shipping. You will need to tell them the shipment date and the UPS/FedEx tracking number. An analysis date will be set at CLCS prior to the end of each cohort. Failure of the specimens to arrive at CLCS prior to this date will result in a delay in processing.
2. Sending a box: When all boxes are packed and ready to be sent, you will need to enter the actual sent date in the Lab Tracking system. Select a box to send in the system. You will be required to enter the sent date and you can add any additional comments if needed. After entry of the sent date, the system will automatically print out a final shipping log and visual map for you to include with the shipment.
3. When the shipping box is packed, make three copies of the shipping log and two copies of the visual map. Keep one copy of each on file at the site, send one copy of each with the specimens to the relevant laboratory, and send one copy of the shipping log only to the CC. Do not send the working drafts.
4. Ship specimens to CLCS and BBI in the large shipping container provided. Obtain sufficient dry ice to fill the cooler. Note: Delay in adding dry ice to the specimens after removal from the freezer will allow specimens to thaw. Insufficient dry ice during shipment will do the same. Any degree of thawing before analysis will damage the specimens and compromise assay results. Please see the packing/shipping instructions provided by the labs. They will send these instructions with the shipping containers. The CLCS will confirm receipt of shipment with the CC.
5. Notify CLCS and BBI before sending each shipment.  
CLCS: Phone: (314) 362-3522 Fax: (314) 362-4782  
BBI: Fax: (301) 208-8829
6. Specimens must be shipped by Federal Express to:  
Dave Gibson  
Core Laboratory for Clinical Studies  
Lipid Research  
4940 Parkview Place  
St. Louis, MO 63110  
Phone: (314) 747-1127  
**-or-**

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Carla Hansen  
BBI - Biotech Research Laboratories  
217 Perry Parkway  
Gaithersburg, MD 20877  
Fax: (301) 208-8829

7. If you have any specimens left that have not been assigned to a box and shipped, print out the following reports from the Lab Tracking system: Central Lab Data Completeness (LAB05) and Central Lab Samples to Discard (LAB06) to verify the remaining samples. The first report will show the samples we are expecting to send and the second will show those samples of non-randomized participants you will need to discard. If there are any additional remaining specimens that you are unable to deal with using the above reports, please contact the Data Manager at the CC.

### *Shipping Specimens for Folate, Carotenoids and Vitamin B-12*

Specimen shippers will be supplied by CDC, and the outside covers will have been covered with clear plastic tape to facilitate the removal of shipping labels. Periodically, we will refurbish these shippers with new outside covers. Fill out the shipping manifest list supplied by CDC itemizing participant's identification numbers, date of collection, clinic name and location, and mailing date for each shipment. Add any pertinent comments regarding condition of specimens such as "inadequate blood draw," "serum specimen hemolyzed," etc. Include one copy of the shipping manifest list with the shipper under the outer lid, and if possible, fax a copy to CDC (770-488-4609) to enable us to track shipments (*please be sure to include the FedEx or UPS shipment number on this copy, as well as the Premier Study Tracking #2000-0030*). Retain a third copy in the clinic for your records. Arrange for shipment by Federal Express or other express courier. Centers are responsible for shipping costs.

When packing the shipment, please follow these instructions:

- (1) Put a layer of several paper towels or sheets of newspaper in bottom of the Styrofoam shipper to act as cushioning. Place at least 10-12, preferably 15, pounds of dry ice in the bottom of the shipper. Do not stint on the dry ice or your specimens could arrive thawed if the shipment is delayed. Chunks or pellets of dry ice are easier to handle than slabs, but do tend to volatilize away more quickly. Add another layer of paper. (Dry ice should never come into direct contact with the plastic bags.)
- (2) Enclose each white box in a large ziplock bag to contain its contents in case of breakage.
- (3) Place the ziplock bag containing the white cardboard box, "right-side up", on top of the paper towels. Fill the remaining space with "bubble"wrap or paper towels or wadded up newspaper to prevent to specimens and dry ice from moving around inside the shipper. The empty white boxes and ziplock bags will be returned to the

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clinics after each shipment.

- (4) Make sure the Styrofoam inner lid is completely closed to preserve cold temperature.
- (5) Place the shipping list on top of the Styrofoam lid. Secure the outer cardboard lid of the shipper firmly with clear or nylon reinforced strapping tape, and attach the Federal Express label. Excessive amounts of tape are not necessary, since the shippers will be returned to each clinic after each shipment. The tops of each new shipper will be covered with the clear tape to make removal of old labels easier.

**Send all shipments to CDC by an overnight express courier on Monday, Tuesday, or Wednesday, and arrange shipment time so that the shipment does not arrive on a weekend or a holiday without advance notice. Specimens should arrive at CDC within 72 hours of shipment.**

Address shippers as follows:

Mr. Charles Dodson  
Centers for Disease Control and Prevention  
4770 Buford Highway  
Bldg. 17 Loading Dock  
Atlanta, GA 30341

Include your clinic address and telephone number on the shipping label and ensure the **Premier Study Tracking # 2000-0030 is noted on the label**. This tracking # is used by CDC to keep track of different study samples arriving at CDC and for linking each study sample to its relevant electronic data file.

Your primary contact persons at CDC for specimens and shipping questions are Charles Dodson and Dan L. Huff. Their contact details are:

Charles Dodson at (770) 488-4305 or fax (770) 488-4541 or e-mail [WCD1@CDC.GOV](mailto:WCD1@CDC.GOV)  
Dan L. Huff at (770) 488-7932 or fax (770)488-4609 or e-mail [dlh1@cdc.gov](mailto:dlh1@cdc.gov)

Notify them when your shipment is being sent to CDC as they need to know when to expect them so it will not be lost /misplaced within CDC.

E-mails should go to both Charles Dodson and Dan L. Huff incase one is away. Be sure to mention the Premier Study and Premier Study Tracking # 2000-0030 in the subject line of any e-mails sent to CDC.

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### ***Central Laboratory Procedures***

#### ***Shipper Receipt at CDC***

Once a shipper arrives at CDC, it will be logged in by the receiving clerk. Specimens will be removed and frozen for short-term storage (several days at most). White storage boxes and new ziplock bags will be replaced in the shipper. Excess labels will be removed carefully, and the shipper will be resealed and returned by surface mail to the originating clinic for reuse. Periodically, new outer cartons will be provided for the Styrofoam inner shells.

All excess serum will be stored at -70 EC until study completion, at which time NHLBI may decide to archive these specimens permanently elsewhere.

#### ***Reporting of Results***

Quality assurance is an important component of reporting of results. If specimens arrive at the CLCS that cannot be analyzed (e.g., not labeled, thawed, insufficient quantity, etc.), the laboratory will notify the clinical site and coordinating center.

The clinical site will be notified of all alert laboratory values (glucose <40 or >400, and total triglycerides >900) as soon as results are available. Alert values are listed in Appendix B.

Prior to release of all results, the values are verified as outlined below. Reference ranges are listed in Appendix B.

- Extreme values are flagged. Assays are repeated and results are verified.
- Delta Checks are reviewed for previous visit comparison. Discrepancies are investigated.

Electronic Data will be transmitted to the sponsor after randomization and after the 6-month and 18-month visits for each cohort.

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### ***Appendix A: 24-Hour Urine Instructions***

#### *Instructions for Standard Weekday Collection*

To collect a 24-hour urine specimen, you will need a plastic sample container (women may also want to use a collection device referred to as a hat). The container should be labeled with your study identification number, the date and time you begin the urine collection and the date and time you complete the collection. It is important to collect all of the urine you pass during the 24-hour collection. However, if you do forget and miss a collection, it is equally important that you indicate how many voidings were missed on the tag at the end of the collection.

Women should use the hat to help collect the sample, place it under the toilet seat, urinate into the hat, and then carefully empty the contents of the hat into the jug. Otherwise, urinate directly into the jug. In the event of a spill, please estimate the amount spilled; write the amount on the recording tag (e.g., "1 tsp spilled"). If you miss a sample, record this on the tag in the place provided.

You will begin your urine collection at the clinic. When you arrive at the clinic, you should void but do not collect this urine. This is the start of your collection period. Beginning with the second urination of the day, collect all urine for the next 24 hours from the time of the discarded urine. Every time you have to urinate, collect the entire sample in the container. For example, the last sample collected should be voided 24 hours after the clinic urination (the times on the recording tag might be 4:00 p.m. start time and 4:00 p.m. stop time, for example) and should also go into the container. Record the date and time of your final urination on the tag of your container. Store the container in a refrigerator or a cooler between voids if possible. Bring your sample into the center as soon as possible after collection is complete.

#### *Special Instructions for Weekend Collection*

Weekend collections should only be done if it is not possible to do the collection on a weekday. Quality control is much improved if the collection starts and ends with a void at the clinic.

Begin the urine collection in the morning. Discard the first void of the day, but mark this as the start time. Write the start date and time on the jug. Beginning with the second urination of the day, collect all urine for the next 24 hours from the time of the discarded urine. For example, if the first void (which is discarded) was at 7:30 am on Sunday, the final void (which is collected) should be at or very close to 7:30 am on Monday.

Every time you have to urinate, collect the entire sample in the container. Record the date and time of your final urination on the tag of your container. Store the container in a refrigerator or a cooler between voids if possible. If more than 24 hours will elapse between the start of the collection, and bringing the jug into the clinic, the jug **must** be refrigerated. Bring your sample into the clinic as soon as possible after collection is complete.

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**Appendix B: Reference Ranges and Alert Values**

*Lipid Profile*

Analyte	Units	Reference Range	Alert Level *	
Total Triglycerides	mg/dL	Desirable: Borderline High: High: Very High:	≤ 200 201-399 400-1000 >1000	>900
Total Cholesterol	mg/dL	Desirable: Borderline High Risk: High Risk:	< 200 200-239 ≥ 240	N/A
LDL Cholesterol	mg/dL	Desirable: Borderline High Risk: High Risk:	< 130 130-159 ≥ 160	≥190
HDL Cholesterol	mg/dL	Desirable: High Risk:	≥ 35 < 35	N/A

*Urine Chemistry*

Analyte	Units	Reference Range	Alert Level*	
Urine Sodium	mmol/24 hr	40-220	N/A	
Urine Potassium	mmol/24 hr	25-125	N/A	
Urine Phosphorous	g/24 hr	0.4-1.3	N/A	
Urine Creatinine	mg/24 hr	Male 800-1800	Female 600-1600	N/A
Urine Urea Nitrogen	g/24 hr	12-20	N/A	
Urine Volume	mL/24 hr	Male 800-1800	Female 600-1600	N/A

*Chemistry and Special Chemistry*

Analyte	Units	Reference Range	Alert Level*
Glucose, Fasting	mg/dL	65-110	>125 or <40
Homocysteine	μmol/L	6.0-16.0	N/A
Insulin, Fasting	μIU/mL	0-23	N/A

**\*Notify participant as soon as alert result comes to the attention of the clinical site.**