

## BLOOD AND URINE COLLECTION

### Background and Rationale

The Mr. OS Dental study involves the collection of approximately 25 mL of blood, and a urine specimen from each participant. Since the study depends on the voluntary return of participants over an extended period of time, every effort must be made to make the entire procedure as easy and painless as possible both for the participants and for the clinical center personnel.

A standard informed consent has been prepared for this study. With regard to laboratory procedures, the consent statement informs study participants that there is a small risk of bruising at the spot on the arm where the blood is taken and that about three to four tablespoons of blood are drawn.

### Equipment and Supplies

#### Sample ID Labels

You will be supplied with sheets of sample ID barcode labels to use for labeling forms, draw tubes, and cryovials. A sample sheet of barcode labels can be found in Appendix 1. All labels on each sheet have the same 6-digit sample ID number (the first two characters identify the clinic: BI = Birmingham, MN = Minneapolis, PA = Palo Alto, PI = Pittsburgh, PO = Portland, SD = San Diego).

There are 17 labels per participant, containing the ID number. There are 5 labels without bar codes, there are to be used for pre-labeling the 2 draw tubes, the urine collection container, and the zip-loc bag for the blood blotter.

There are 12 labels with bar codes, including one named "Specimen Collection Form" which is placed TELEform. This process of matching the participant-specific ID (**already on the form brought to the lab**) to the sample-specific ID barcode is crucial to being able to use the data collected from laboratory tests.

Finally, there are 11 labels intended for labeling cryovials, each with an ID number and barcode.

### Blood Collection Trays and Tubes

Blood drawing trays are prepared in advance for the following day. Each tray is stocked with a full supply of blood drawing equipment for 8-12 participants and holds an ice bath and the individual blood collection tube rack for each participant. Several racks will also be prepared to hold various plastic tubes and vials for the final serum, urine and whole blood aliquots sent to Biomedical Research Institute (BRI) for storage.

### Blood Collection Tray

The collection tray itself is made of hard plastic which is unbreakable and can be easily cleaned. The tray has ten individual compartments, which are filled with the following supplies:

Alcohol swabs	Smelling salts
Band-Aids	Scissors
Gauze	Adhesive tape
Tourniquets (2)	Pencils/pens
Vacutainer holders	Latex gloves
Needle/sharps container	Aluminum foil
Styrofoam ice bath filled ~10 min before draw	
21G Butterfly needles	

### **Blood Collection Rack: Labeling and Setup**

A separate tube rack containing the necessary draw tubes is set up for each participant. This rack will fit into the blood collection tray. The blood collection tubes and urine cup should be pre-labeled with sample ID labels. The “Specimen Collection Form” label should be clipped to the corresponding blood collection tray. The label for the blood blotter should be clipped to the corresponding aliquot rack.

### **Description of Blood Collection Tubes**

Each draw tube is color coded to aid in handling.

Draw tubes # 1 and # 2 are 10 mL siliconized red stoppered tubes used to collect serum. These tubes contain no anticoagulant, so the blood will clot to form serum. After drawing, the blood is allowed to clot at room temperature for 40-45 minutes (Maximum = 90 minutes). After blood collection, Tube # 2 is wrapped in aluminum foil to protect the sample from light. One 1mL aliquot will be collected for analysis of vitamin D (cryovials # 10). Cryovials # 1- #9 will be used to collect serum for future analyses.

Tubes # 2 is a 4 mL lavender stoppered tubes containing 15% EDTA as the anticoagulant. After drawing, the tube is mixed and immediately placed on ice. The first ½ mL aliquot from this tube will be used in creating the blood blotter. The remaining whole blood from this draw will be discarded. Please note, although the remaining blood will be discarded, the tube must be filled as completely as possible to ensure the correct ratio of EDTA to blood in the collection tube.

### **Safety Issues and Exclusions**

#### **Precautions for Handling Blood Specimens**

In accordance with the OSHA regulations on blood borne pathogens (see Appendix 7 for complete OSHA regulations), we recommends the following laboratory safety protocol for the field center laboratories:

- Non-permeable lab coats, latex gloves, and face shields should be used when handling any blood in any situation where splashes, spray, spatter, or droplets of blood may be generated and eye, nose, or mouth contamination can be reasonably anticipated.
- 'Universal Precautions' should be followed when handling any blood products.
- Contaminated needles and sharps shall be immediately placed in a puncture-resistant, leakproof container. Never recap or break needles.
- Hepatitis B vaccine should be offered to all unvaccinated technicians handling blood and documentation of vaccination or technician's declining to be vaccinated should be kept

### **Participant Precautions and Exclusions**

#### **Specimen Collection Worksheet**

Following the questionnaire format on the Specimen Collection Worksheet, each participant is asked whether they have a bleeding disorder before the blood is drawn. If they have had any problems with excessive bleeding or bruising at a venipuncture site, use your own judgment to decide whether or not a clinic physician or nurse supervisor should be consulted.

There is no action to take if the participant has been told they have a coagulation disorder.

If the participant has experienced fainting spells during phlebotomy ask the participant the frequency of fainting spells. If the participant frequently faints, again, use your own judgment to determine whether or not a consultation with the clinic physician or nurse supervisor is necessary. Provide smelling salts, basin, and a cold cloth if needed. See section below on precautions when a participant feels faint.

#### **PRECAUTIONS WHEN A PARTICIPANT FEELS FAINT OR LOOKS FAINTS FOLLOWING THE BLOOD DRAWING**

- Have the participant remain in the chair, if necessary have them sit with their head between their knees.
- Provide the participant with a basin if they feel nauseated.
- Have the participant stay sitting until their color returns and they feel better.
- Place a cold wet cloth on the back of the participant's neck.
- If the participant faints, use smelling salts to revive by crushing the ampoule and waving it under the participant's nose for a few seconds.
- If the participant continues to feel sick, contact a medical (nursing) staff member who will advise you on further action.

#### **Participant Refusal to Provide Urine Sample or Refusal of Phlebotomy**

Rarely, a participant will refuse to provide a urine sample or will refuse phlebotomy. Please keep a list of MrOS ID #s of any of these participants and identify which test they refused.

## **Subject and Exam Room Preparation**

### **Phlebotomy Room**

The blood drawing should take place in an isolated room or where room dividers can separate participants. The room should be equipped with all of the necessary blood drawing supplies. A separate counter or work table should be equipped with all of the materials and vials that are used in the blood handling and processing. The centrifuge, refrigerator, and freezer should be nearby.

### **Preparation for Phlebotomy**

Preparation for phlebotomy is done in the following manner. Early morning, before any participants arrive:

- Check to make sure that blood collection tray is properly equipped.
- Check that each vacutainer tube and urine cup is properly labeled with ID labels and numbered 1-3.
- Check that the sample processing station is properly equipped
- Make sure the phlebotomy room is tidy and stocked with extra smelling salts, basin, and disposable wash cloths.

### **Preparation of Participants for Urine Collection**

Urine will be collected on all participants whenever possible. Collection is considered a 'spot' urine collection. Encourage the participant to drink water prior to the clinic visit as this will make the phlebotomy and urine collection easier.

It is preferred that the urine is collected prior to the blood draw, although urine collection later in the visit is acceptable.

Participants may urinate directly into a specimen collection container. Containers for routine specimens should be chemically clean, should hold about 3 oz in volume, and must have a tight-fitting lid to prevent leakage during transportation to the lab.

### **Instructions for Participants:**

The participant's privacy should be assured. He should perform the following steps:

1. Have all equipment ready.
2. Void directly into the collection container until half full.
3. Carefully seal the cap of the container so that it is tight and leak proof.

## **Preparation of Participants for Phlebotomy**

It should be stressed that this study requires the voluntary cooperation of the participants. These people are donating both time and blood on a purely voluntary basis, with no reward other than the knowledge that they are contributing to progress in medicine. Thus, the whole experience must be made as pleasant as possible. Two tubes of 10 mL of blood are collected. Each tube contains only 2 teaspoons of blood. The phlebotomist may also assure participants that they donate more than 10 times as much blood (450 mL) when they donate a unit of blood.

## **Detailed Procedures**

### **Forms**

The purpose of this form is to facilitate the collection of serum and urine samples from. The collection must be done in a rapid and efficient manner, with maximum protection for the participant. In addition, the process must facilitate the monitoring of phlebotomy and other quality assurance parameters. All forms must be completed in ink.

The participant will arrive at the phlebotomy station with their with Mr. OS participant ID # already filled in on their Specimen Collection Form. Make sure that the draw tubes are labeled with the correct MrOS ID number. It is very important that the participant's MrOS ID number matches the ID numbers listed on each of the draw tubes and cryovials. There will be a small sheet of labels clipped to the rack of vacutainers. There is a "Specimen Collection Form" label which should be affixed to the Specimen Collection Form. This should be done before drawing any blood, to insure that this critical task is not forgotten.

There are two parts to the Specimen Collection form; the first questions determine if a participant has provided any samples, and the other questions determine laboratory inventory.

### **Return Visit Aliquots**

Occasionally, participants return to the clinic just to give a urine sample or have a blood draw. Follow the procedure as for a regular clinic visit. Be sure to fill out both forms with the header information including the Mr. OS ID #, Acrostic, Date Form Completed, and Staff ID #. Double check to make sure that the MrOS ID listed on the forms matches the ID numbers on the draw tubes and cryovials.

## **Phlebotomy**

### **General**

Blood drawing is standardized for the sitting position.

The venipuncture is performed with a 21 gauge butterfly needle with 12 inches of plastic tubing between the venipuncture site and the blood collection tubes. A 23 gauge needle may be used, if necessary, for a difficult draw. The butterfly has a small, thin-walled needle, which minimizes trauma to the skin and vein. The use of 12 inches of tubing allows tubes to be changed without

any movement of the needle in the vein. If the participant is concerned about the venipuncture, he may be reassured to know such care is taken. The participant should be given enough time to feel comfortable both before and after the blood collection. In many cases the most memorable part of the experience for the participant will be the contact with the technician who draws the blood and their general attitude and competence.

If the participant is nervous or excited, the technician briefly describes the procedure. Sample script: *"I am going to be drawing about 4 teaspoons of blood. We hope to be able to use the results of these tests to better understand health and disease in older people."*

### **Handling participants who are extremely apprehensive about having blood drawn**

*Do not under any circumstances force the participant to have blood drawn.* It may help to explain to the participant that the blood drawing is designed to be as nearly painless as possible. It is sometimes best to let the participant go on with another part of the visit. It may also be helpful to have the participant relax in the blood drawing chair just so the phlebotomist can check the veins in the participant's arms, without actually drawing blood. If the participant has "good veins" the phlebotomist can reassuringly say, "Oh, you have good veins; there should be no problem." Elderly patients are often aware of the difficulty they pose to phlebotomists and should receive extra consideration and detailed explanations as required.

### **Venipuncture Procedure**

- Wear Latex gloves and a lab coat.
- Apply tourniquet.
- Examine participant's arms for the best site for venipuncture. Generally the antecubital vein is preferred, if feasible. Release tourniquet.
- Cleanse venipuncture site. Prepare area by wiping with alcohol swab in a circular motion from center to periphery. Allow area to dry.
- Reapply tourniquet and note the time on the Specimen Collection Form.
- Grasp the participant's arm firmly, using your thumb to draw the skin taut. This anchors the vein. The thumb should be 1 or 2 inches below the venipuncture site.
- With the needle bevel upward, enter the vein in a smooth continuous motion.
- Make sure the participant'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 participant make a fist with the opposite hand and place it under the elbow for support.
- Grasp the flange of the vacutainer holder and push the tube forward until the butt end of the needle punctures the stopper, exposing the full lumen of the needle.
- Note the blood flow into the first collection tube. If blood is flowing freely, the butterfly needle can be taped to the participant's arm for the duration of the draw. If the flow rate is very slow, the needle may not be positioned correctly.
- Keep a constant, slight forward pressure (in the direction of the needle) on the end of the tube. This prevents release of the shutoff valve and stopping of blood flow. Do not vary pressure or reintroduce pressure after completion of the draw.
- Fill each vacutainer tube as completely as possible; i.e., until the vacuum is exhausted and blood flow ceases. If a vacutainer tube fills only partially, remove the vacutainer and attach one of your extra, backup tubes of the same type without removing the needle from the vein.

- When the blood flow ceases, remove the tube from the holder. The shutoff valve re-covers the point, stopping blood flow until the next tube is inserted.
- As the tube is removed, mix by gently inverting.

### **Removing the Needle**

- 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 into puncture-proof sharps container.
- Have the participant hold the gauze pad firmly for one to two minutes to prevent a hematoma.

### **Bandaging the Arm**

Under normal conditions:

- Slip the gauze pad down over the site, applying mild pressure.
- Apply an adhesive or gauze bandage over the venipuncture site after making sure that blood flow has stopped.
- Tell the participant to leave the bandage on for at least 15 minutes.

If the participant continues to bleed:

- Apply pressure to the site with a gauze pad. Keep the arm elevated until bleeding stops.
- Wrap a gauze bandage tightly around the arm over the pad.
- Tell the patient to leave the bandage on for at least 15 minutes.

### **Completing the Blood Drawing Procedure**

- Dispose of needle and tubing in the appropriate biohazard needle sharps containers.
- Complete Specimen Collection form.
- Clean up the venipuncture area (if necessary).
- Bring blood collection tray to the processing area with the filled vacutainer tubes.

### **Procedures for Difficult Draw**

If a blood sample is not forthcoming, the following manipulations may be helpful.

- If there is a sucking sound, turn needle slightly or lift the holder in an effort to move the bevel away from the wall of the vein.
- If no blood appears, move needle slightly in hope of entering vein. Do not probe. If not successful, release tourniquet and remove needle. A second attempt can be made on the other arm.
- Loosen the tourniquet. It may have been applied too tightly, thereby stopping the blood flow. Reapply the tourniquet loosely. If the tourniquet is a velcro type, quickly release and press back together. Be sure, however, that the tourniquet remains on for no longer than two minutes at a time.

- DO NOT attempt a venipuncture more than twice unless a participant encourages you to do so.
- Reassure the participant that the inability to obtain a clean venipuncture is not any sign of a medical problem on their part.
- If venipuncture is unsuccessful, all efforts should be made to have the participant rescheduled at a later date, preferably with a different Field Center phlebotomist. If at least one red stoppered tube (for serum) AND the lavender topped tube (for whole blood) are collected, then a return visit is not necessary.

### **Other Possible Problems**

1) Collection tube does not fill: First, try another tube of the same type. Partial tubes for serum are okay, but will result in a reduced number of aliquots.

### **Quality Assurance**

#### **Training Requirements**

Clinical experience with phlebotomy is mandatory. Additional training should include:

- Read and study manual
- Attend Mr. OS training session on techniques (or observe procedure by experienced examiner)
- Discuss problems and questions with local expert or QC officer

#### **Certification Requirements**

- Complete training requirements
- Explain what to do for difficult venipuncture
- Recite measures to take for fainting participant
- Conduct phlebotomy on volunteer or participant while being observed by QC officer using QC checklist

#### **Quality Assurance Checklist**

Preparation:

- Blood collection trays properly prepared
- Blood draw tubes properly labeled
- Proper instructions given for urine collection
- Hepatitis B vaccination given or offered to all personnel handling blood

Venipuncture properly carried out:

- Script properly delivered
- Non-permeable lab coats, gloves, and face shields used
- Preparation of venipuncture site correctly done
- Venipuncture smoothly done



- Collection tubes at least 2/3 full
- Tourniquet removed at 2 minutes
- Needle removed and arm bandaged correctly
- Needle and tubing appropriately dispose

Specimen Collection form properly filled out:

- Sample ID barcode label affixed Time at start of venipuncture entered
- Quality of venipuncture checked
- Urine collection time recorded

## LAB PROCESSING GUIDELINES

### Equipment and Supplies

A complete supply list with ordering information can be found in Appendix 6. Necessary supplies include:

- Horizontal centrifuge
- -20° Freezer space is required
- Dry Ice
- Pipets and tips: 1.0 mL volumes
- Lab coat and gloves
- Biohazardous waste disposal container
- Balance tubes for the centrifuge
- Lab mat
- 10% bleach solution
- Freezer boxes with 9 x 9 cell grid (for 1.8 mL serum and urine cryovials)
- Rubber bands
- Aluminum foil

### Sample ID Labels

Each cryovial label also has a 2-digit vial # (01 to #11) that serves as a unique identifier for each cryovial with MrOS ID #. The labels for cryovials have bar codes to help track the repository. See Appendix 4 for proper orientation of the barcode label.

Beneath the ID number, cryovial labels also have lines of text: vial #, cap color, and type of specimen (serum, urine). This line of text is intended to increase accuracy in the labeling and filling of the cryovials.

There are a total of 17 labels for each participant. Eleven of these labels contain barcodes; these labels should be properly affixed to the appropriate cryovial.

### Laboratory Room Preparation

All items required for sample processing should be on hand before beginning processing.

Aliquot racks will be set up to correspond to each blood collection tube rack. Rack setup is completed the previous day. All tubes and vials are labeled with sample ID bar codes (see Label Orientation diagram in Appendix 2) and arranged in appropriate working order.

### Detailed Procedures

#### Processing

The draw tube may be held at room temperature for up to 90 minutes. Personal protective equipment (non-permeable lab coats, double-gloves with at least one latex pair, splatter shields) **MUST BE** worn for processing.

### **Immediate Processing**

The draw tube must remain at room temperature for a minimum of 40 minutes. Room temperature is 21° C (the range of 15.5° → 23.5° is acceptable), 70° F (the range of 60° → 75° is acceptable). Allowing the tube to stand longer may increase the yield of serum. The maximum allowable time before centrifugation is 90 minutes.

If the urine specimen cannot be immediately, place the filled urine cup into the refrigerator until the processing can begin. UV light may damage the specimen.

### **Aliquots per Sample Type**

The following is a summary of the processing. Detailed instructions follow (volume indicates sample size, not cryovial size). Please see Appendix 2 for a flow chart that illustrates this graphically.

**Serum:** The serum from the 10 mL tubes is spun and then aliquoted into 9 aliquots of various amounts which are placed in 1.8 mL cryovials.

Cryovial #	Cap Color	Amount to aliquot
1	clear	1.0 mL
2	clear	1.0 mL
3	clear	1.0 mL
4	blue	<b>0.5 mL</b>
5	blue	<b>0.5 mL</b>
6	brown	1.0 mL
7	clear	1.0 mL
8	clear	1.0 mL
9	clear	1.0 mL

**Urine:** The urine is aliquoted into two 1 mL samples, which are contained in 1.8 mL cryovials, numbered cyovial #10 and #11.

### **Centrifugation of Serum Samples**

After 40-45 minutes (maximum 90 minutes) the draw tubes should display a clot.

Use a horizontal centrifuge; angle heads are not satisfactory.

Centrifuge the blood for 10 minutes at room temperature at a setting known to yield a relative centrifugal force (RCF) of at least 1000 x g at the bottom of the tubes. The table below gives those combinations of centrifuge speed in revolutions per minute (rpm) and rotating radius (r) that will yield an RCF value of 1000 x g. RPM should be read from a tachometer or rev counter when the centrifuge is normally loaded. Radius (r) is measured in centimeters from the center of the rotor shaft to the bottom of the vacutainer tube when the tube is in a horizontal position.

r (cm)	12	14	16	18	20	22.5	26
rpm	2800	2600	2400	2250	2100	2000	1900

Do not use a brake to slow down the centrifuge.

### **Making Serum Aliquots**

Allow the centrifuge to come to a complete stop. Carefully remove the tubes from the centrifuge, being careful not to shake the tubes, and place them on ice. Please note that the cryovials will contain different amounts of serum for storage; vials #4 and #5 have 0.5 mL of serum, remaining vials have 1.0 mL of serum.

If the volume requirement (i.e. 1.0 mL for vials #1-3 and 6-9; 0.5 mL for vials 4 and 5) is met for the cryovial, fill in the circle in the column titled "Complete" on the Specimen Collection Form. If the tube is only partially filled (less than the volume requirement, NOT less than 1.8 mL), mark the circle labeled partial (for partial). If a sample is hemolyzed, mark the circle marked hemolyzed. If the serum is reddish in color, determine if it is hemolyzed or simply contaminated with red blood cells. One can tell the difference by recentrifuging the vacutainer tube. This will pellet any contaminating red cells and the serum will clear. If the sample is hemolyzed the red color will remain in the serum. If the tube is both hemolyzed and partially filled, mark the circle labeled hemolyzed/partial. If you are unable to fill the designated cryovial, fill in the circle labeled "not filled."

### **Making Urine Aliquots**

- Pipet 1.0 mL of urine into the two 1.8 mL capped cryovials and place on ice. (Yellow cap, cryovials # 10 and #11)
- Discard the remaining urine.

### **Freezing**

Upon completion of the processing steps, serum and urine aliquots must be frozen at -20° or on dry ice within 30 minutes.

After aliquoting is complete, the rack containing the cryovials should be removed from the ice bath and placed upright in the freezer at -20° C (or on dry ice or colder) for at least half an hour (preferably until the end of the day). Make sure the aliquots are not wet when placed in the freezer. If a freezer is not immediately available, place the rack of samples on dry ice.

### **End of the Day Procedures**

- Frozen cryovials in racks are packaged into freezer boxes by numeric order of cryovials per participant. The cryovials will be placed into the boxes with the 9 x 9 grid. Do not leave empty spaces in the boxes. Samples from one participant may overlap into two boxes, and box will have samples from more than one participant.
- Re-stock blood collection trays with supplies.
- Label the next day's draw tubes and cryovials.
- Arrange draw tubes and aliquots in their proper racks.
- Wipe down all work areas with 10% Clorox solution.

### **Shipping the Blood Samples**

#### **General**

Frozen blood and urine samples are shipped *bimonthly* to BRI by Federal Express overnight delivery. Do not ship on Thursdays or Fridays to avoid delivery of shipments during a weekend.

Shipments to BRI are charged to your local Federal Express account number.

This shipping protocol follows the procedures mandated by the International Air Transport Association's Dangerous Goods Regulations-Packaging Instructions 650 and 904.

#### **Methods for shipping frozen samples**

The frozen blood cryovials are already packaged in prelabeled freezer boxes and stored in the -20° C freezer by consecutive box number.

Complete a grid detailing the contents of the shipment, including MrOS ID #, vial #, and cryovial cap color codes (e.g., Bl (for blue), Cl (for clear), Br (for brown), and Y (for yellow)). Please see Appedix 5 for a sample grid.

Do not leave empty spaces in the boxes. Each box for shipment will contain samples from more than one participant. Additionally, a participant's samples may be shipped in two separate boxes.

Samples should be prepared for shipping as follows:

- Wrap each freezer box in paper towels to absorb possible leakage. Put a rubber band around the towel-wrapped box or bag. Using two rubber bands, put a rubber band in each direction (horizontally and vertically), forming a cross with the rubber bands.
- Put the individual freezer boxes containing the samples into a leakproof zip-lock plastic bag. Seal the zip-lock bags.
- Line the styrofoam mailer with absorbent material (e.g., paper towels).
- Place approximately one third of the dry ice on the bottom of the mailer.

- Carefully place the freezer boxes into the styrofoam mailer. Place no more than a total of 4 L of sample into the styrofoam shipping container. Use two or more styrofoam mailers for the BRI shipment when necessary. (In this case, label the mailers “1 of 2” and “2 of 2”).
- Place the remaining dry ice (approximately 7 - 14 lbs. total) on top and around the samples to fill the styrofoam container.
- Enclose the styrofoam container in the outer cardboard sleeve.
- Enclose the completed grid with cryovial information and copies of the Specimen Collection Forms and Laboratory Processing Forms.

The BRI mailing address is:

Chris Kennell  
Biomedical Research Institute  
12264 Wilkins Avenue  
Rockville, MD 20852

FAX the following information to BRI at (301) 770-9811 when a shipment is sent:

Date of shipment  
Expected arrival date  
Number of styrofoam mailers shipped  
FedEx airbill number

### **Quality Assurance**

### **Training Requirements**

Clinical experience with processing of blood samples is strongly recommended. Additional training should include:

- Read and study manual
- Attend Mr. OS training session on techniques (or observe processing by experienced examiner)
- Discuss problems and questions with local expert or QC officer

### **Certification Requirements**

- Complete training requirements
- Recite shipping schedule.
- Process samples from volunteer or participant while being observed by QC officer using QC checklist.

**Quality Assurance Checklist**

## Preparation

- Aliquot racks correctly set up
- Cryovials correctly labeled
- Hepatitis B vaccination given or offered to all personnel handling blood
- Non-permeable lab coats, gloves, and face shields used

## Processing whole blood and urine

- Blood blotter completed
- Urine correctly aliquoted

## Processing serum tubes

- Centrifuge correctly balanced with water tube(s)
- Serum correctly aliquoted

## Freezing

- Rack placed upright in -20° C freezer or samples placed on dry ice

## End of day procedure

- Frozen aliquots removed from rack and placed in appropriate freezer boxes
- Freezer boxes correctly labeled

## Shipment procedures -- dry ice

- Freezer boxes correctly wrapped -- absorbent material, rubber band, and zip-lock bag
- Styrofoam mailers correctly packed -- absorbent material, dry ice, top sealed with tape
- Grid and forms included in package
- Styrofoam mailer sealed in cardboard sleeve
- FedEx airbill correctly filled out
- Labels correctly affixed