

## LAB PROCESSING GUIDELINES

### 1. Background and Rationale

The Mr. OS study involves the collection of approximately 60 mL of blood from participants. The blood is collected in two types of tubes for specialized processing of different blood components. After processing, the specimens will be aliquoted into cryovials to be sent to Biomedical Research Institute (BRI) to store for later analyses.

### 2. Equipment and Supplies

A complete supply list with ordering information can be found in Appendix 1. 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

#### 2.1 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 2. 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 is 1 barcode label with the ID number, with the words "Laboratory Processing Form," which is placed on the Laboratory Processing Form (Appendix 6). This process of matching the participant-specific Mr. OS Enrollment ID # (**already on the form brought to the lab by the participant**) to the sample-specific ID barcode is crucial to being able to use the data collected from laboratory tests.

Each cryovial label also has a 2-digit vial # (01 to 33) that serves as a unique identifier for each cryovial with Mr. OS Enrollment ID #. The labels for cryovials have bar codes to help track the repository. See Appendix 2 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, whole blood, urine). This line of text is intended to increase accuracy in the labeling and filling of the cryovials. The cryovial cap information is coded (C= clear, Br= brown, etc.). Complete codes can be found at the bottom of the Laboratory Processing form.

There are a total of 45 labels for each participant. Six are to be used for pre-labeling the 6 draw tubes, 2 for the nail samples, and one for the blood blotter. There is one label for the urine specimen cup. These labels have no barcode, and they have 1-3 lines of text indicating which specimen container they are intended for, including the stopper color and volume, if applicable.

### 3. Safety Issues and Exclusions

#### 3.1 Precautions for Handling Blood Specimens

In accordance with the OSHA regulations on blood borne pathogens (see Appendix 2 for complete OSHA regulations in Specimen Collection Operation Manual), the following laboratory safety protocol is recommended 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.

#### **4. Laboratory Room Preparation**

##### **4.1 Preparation for Processing**

All items on the Sample Processing Checklist (Appendix 3) 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 (see Aliquot Rack Setup diagram in Appendix 4).

#### **5. Detailed Procedures**

##### **5.1 Processing**

###### **5.1.1 General**

Draw Tube #2 will be mixed and immediately placed on ice. The other draw tubes may be held at room temperature for up to 90 minutes (serum tube # 1, 3, 4, 5, 6). After blood collection, draw Tube # 1 should be wrapped in aluminum foil because two mLs of serum from this tube tested for light-sensitive vitamin D. Personal protective equipment (non-permeable lab coats, double-gloves with at least one latex pair, splatter shields) MUST BE worn for processing. A flow chart is included in Appendix 5 to diagram this process.

It is possible that not all tubes will be collected due to problems with phlebotomy. During processing, work in the order specified and make as many aliquots as possible while meeting the volume requirement of each cryovial

How to fill out the Lab Processing Form for each cryovial:

<b>Condition of tube</b>	<b>Indication on form</b>
Filled; not hemolyzed	Ok
Filled; hemolyzed	H (for hemolyzed)
Partially filled, not hemolyzed	P(for partial)
Partially filled, hemolyzed	B(for both)
Not filled	Not filled

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

Each draw tube is color coded to aid in handling.

Draw Tubes # 1, 4, 5 are 10 mL siliconized red stoppered tubes used to collect serum. After blood collection, draw Tube # 1 will be wrapped in aluminum foil. All of 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). Two 1mL aliquots from the aluminum-wrapped tube (draw Tube # 1) will be placed in aluminum foil-wrapped cryovials for analysis of vitamin D (Brown cap, cryovials # 13-14). The remainder of serum from these tubes will be used for archiving.

Draw Tube # 2 is a 10 mL lavender stoppered tubes containing 15% EDTA as the anticoagulant. After drawing, the tube is mixed and immediately placed on ice. A ½ mL quantity of whole blood will be used for the blood blotter. The remaining whole blood will be separated into two 5mL aliquots by carefully pouring the contents into 5 mL aliquots and shipped as whole blood to BRI. The whole blood is used for analysis of DNA.

Draw Tubes # 3, 6 are 10 mL siliconized red stoppered tubes used to collect serum. These tubes contains 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). These tubes are collected at least 20 minutes apart (the maximum time between each collection is 40 minutes), each tube will clot, be spun and aliquoted into five 1 mL aliquots.

### **5.1.3 Immediate Processing**

Upon reaching the blood processing station, remove the blood drawing rack and ice bath containing tubes from the blood collection tray. The ice bath should contain draw tube #2.

Draw Tube # 1, 3, 4, 5, 6 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). Please note that tubes # 3, 6 will come to the blood processing station in 20 minutes intervals. Allowing the tubes to stand longer may increase the yield of serum. The maximum allowable time before centrifugation is 90 minutes. Note the time that serum processing on the Laboratory Processing form.

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.

#### **5.1.4 Aliquots per Sample Type**

The following is a summary of the processing. Detailed instructions follow (volume indicates sample size, not cryovial size).

**Serum:** The serum from 10 mL tubes # 1, 4, 5 is aliquoted into fourteen 1.8 mL cryovials for archival. The first two 1.0 mL aliquots are placed in two 1.8 mL aluminum foil-wrapped cryovials. The remainder of the serum will be separated into 1 mL aliquots, which are placed in 1.8 mL cryovials.

The total number of aliquots is: 14

14 x 1.0 mL

2 (Brown cap, cryovial # 13-14)

12 (Clear cap, cryovial # 15-26).

The serum from 10 mL tubes # 3, 6 are spun and then each aliquoted into a five 1.0 mL aliquots, which are placed in 1.8 mL cryovials.

The total number of aliquots is: 10

5 x 1.0 mL (Green cap, cryovial # 01-05)

5 x 1.0 mL (Purple cap, cryovial # 06-10)

**Whole Blood:** ½ mL of whole blood from 10 mL tube #2 will be used for the blood blotter. The remaining whole blood will be separated by pouring whole blood into two 5 mL cryovials.

½ mL → blood blotter

The total number of aliquots is: 2

2 x ~4.5 mL (Orange cap, cryovial # 11-12)

**Urine:** The urine is aliquoted into seven 1 mL samples, which are contained in 1.8 mL cryovials.

The total number of aliquots is: 7

7 x 1.0 mL (Yellow cap, cryovial # 27-33)

A detailed listing of all aliquots and color codes can be found on the Laboratory Processing Form.

**5.1.5 Centrifugation of Serum Samples**

Draw Tubes # 1, 3, 4, 5, 6 should be left at room temperature for at least 40-45 minutes (maximum 90 minutes) after they are drawn. The tubes should display a clot by this time.

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.

### 5.1.6 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.

Serum (Tubes # 1, 3, 4, 5, 6)

#### 10 mL Tubes # 1, 4, 5

Aliquots: 2 x 1.0 mL serum use aluminum wrapped 1.8 mL cryovial  
(Brown cap)

12 x 1.0 mL serum use 1.8 mL cryovial (Clear cap)

#### 10 mL Tube # 3

Aliquots: 5 x 1.0 mL serum use 1.8 mL cryovial (Green cap)

#### 10 mL Tube # 6

Aliquots: 5 x 1.0 mL serum use 1.8 mL cryovial (Purple cap)

If the volume requirement is met for the cryovial, fill in the circle in the column titled "Ok" on the Laboratory Processing Form. If the tube is only partially filled, mark the circle labeled P (for partial). If a sample is hemolyzed, mark the circle marked H. 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 B (for both.) If you are unable to fill the designated cryovial, fill in the circle labeled "not filled."

### 5.1.7 Preparing Whole Blood Blotters

Procedures:

- a) Place barcode label on the back of a 3 in. by 3 in. piece of filter paper.
- b) Decant the ½ mL of whole blood from draw Tube # 2 onto the filter paper.  
Hold up a corner of the filter paper while decanting so that the blood doesn't

- soak through to the paper towel. The Whatman bloodstain cards will have four circles intended for use as a reference. However, the blood can simply be dropped into the center of the card, and need not be applied within the circles.
- c) Place the whole blood/filter paper on a paper towel and allow to air dry.
  - d) When the blood is completely dry, place the numbered filter paper in a ZipLoc™ plastic bag. The plastic bag should contain 2 tsp. Drierite. Store about 50 pieces of filter paper in each plastic bag, clipped (plastic clip) together in ID order. Record the ID numbers contained in each ZipLoc™ bag on a piece of paper taped to the inside of the bag and visible from outside.
  - e) Store the plastic bags in the airtight plastic container along with 1 Tbsp Drierite for each bag. Store the plastic container in the refrigerator.
  - f) Monitor the Drierite in the bags and the plastic container each week. If the Drierite changes from blue to pink-red in color, replace it with a fresh sample. The Drierite may be recycled by warming it in an oven until it turns blue again.
  - g) The blood blotter will be shipped to BRI in two batches. The first batch will be shipped around the end of March 2001. The second batch will be shipped to BRI once all baseline visits have been completed.

### **5.1.8 Making Whole Blood aliquots**

Keep the collection tube (#2) on ice until aliquoting can occur. After preparing the blood blotter, carefully pour the remaining whole blood sample into two 5 mL cryovials. (Orange cap, cryovial # 11-12)

### **5.1.9 Making Urine Aliquots**

- Pipet 1.0 mL of urine into seven 1.8 mL capped cryovials and place on ice. (Yellow cap, cryovials # 27-33)
- Discard the remaining urine.



### 5.1.10 Freezing

Upon completion of the processing steps, serum, whole blood, 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.

### 5.1.11 Completed forms

The completed Specimen Collection and Laboratory Processing Forms can be set aside in a daily work folder. These forms are copied and then the originals are faxed into the data system and the Coordinating Center and filed at each Field Center. The copies of these forms are to be included with shipment of specimens to BRI. Be sure the participant's Mr. OS Enrollment ID# and acrostic, the sample ID, and the staff ID are legible on the copies (e.g., not cut off by the copier).

## 5.2 End of the Day Procedures

- Frozen cryovials in racks are packaged into freezer boxes by numeric order of cryovials per participant. The 1.8 mL cryovials are placed in separate boxes from the 5 mL cryovials due to their different heights. The 1.8 mL cryovials will be placed into the boxes with the 9 x 9 grid. The 5 mL cryovials will be placed into a separate box with an 9 x 9 grid as well. The boxes will be filled and be shipped at different rates (the box with the 5 mL tubes will be shipped less often.) 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.

### 5.3 Summary of Processing Time Limitations

From end of venipuncture to start of processing:

- |    |             |            |
|----|-------------|------------|
| 1. | Serum 10 mL | 90 minutes |
| 2. | EDTA 10 mL  | 15 minutes |

Once serum centrifuged, maximum time before aliquoting: 15 Minutes

After aliquoting samples, freeze within 30 minutes.

### 5.4 Shipping the Blood Samples

#### 5.4.1 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.

#### 5.4.2 Methods for shipping frozen samples

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

Complete a grid detailing the contents of the shipment, including Mr. OS Enrollment ID #, vial #, and cryovial cap color codes (e.g., Br, C, G, O, Pu, Y).

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

## 6. Quality Assurance

### 6.1 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

## 6.2 Certification Requirements

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

## 6.3 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
- Whole blood correctly aliquoted
- Urine correctly aliquoted

### Processing serum tubes

- Time checked to ensure that draw tubes # 1, 3, 4, 5, 6 has stood at room temperature for at least 40 minutes, maximum 90 minutes
- Draw Tubes # 1, 3, 4, 5, 6 centrifuged for 10 minutes at appropriate speed
- Centrifuge correctly balanced with water tube(s)
- Serum correctly aliquoted
- Cryovial # 13, 14 wrapped in aluminum foil

#### Freezing

- Rack placed upright in -20° C freezer or samples placed on dry ice
- Store 1.8 mL cryovials in freezer boxes with 9 x 9 grid and 5 mL cryovials in separate freezer boxes with 9 x 9 grid

#### End of day procedure

- Specimen Collection and Laboratory Processing forms placed in daily work folder
- 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

## APPENDIX 1 Mr. OS Cryovials and Processing Equipment

Vendor: Fisher Scientific (800) 766-7000

<b>Cryovials/Caps</b>	<b># per participant</b>	<b>Sample Type</b>	<b>Fisher Catalog #</b>
1.8 mL	31	Serum, urine	12-565-171N
5 mL Corning	2	Whole blood	09-761-74
Cap-brown	2	Serum-Vit. D	12-565-251
Cap- green	5	Serum for sex hormones-draw 1	12-565-245
Cap- purple	5	Serum for sex hormones-draw 2	12-565-254
Cap- yellow	7	Urine	12-565-246
Cryovial Racks	1 (can be reused)	All	07-200-618
<b>Other</b>	<b># per participant</b>	<b>Sample Type</b>	<b>Fisher Catalog #</b>
Transfer pipets (built-in bulbs)	3 or 4	All	13-711-5A
Cryotube storage boxes	NA	All	11-678-24B
81 cell cyrotube insert	NA	All	13-989-218
Urine collection cup (3 oz)	1	Urine	02-544-3
<b>Blood Collection</b>			
Vacutainer Blood Collection Set 21 G 3/4 "	1		02-664-1
Vacutainer Blood Collection Set 23 G 3/4 "	optional		02-664
Needle holders	1		02-665-110

## Collection Tubes - Vacutainer brand

<b>Tube#</b>	<b>Type</b>	<b>Volume</b>	<b>Description</b>	<b>Fisher Catalog #</b>
<b>1,3,4,5,6</b>	Serum	10 mL	No additive, silicon coated	02-685A
<b>2</b>	EDTA	10 mL	With 15% EDTA	02-683-84

Blood Blotter Supplies:

Filter paper: FC180 FTA Classicard (treated), 1 per ppt. Approximate cost: \$1 per card


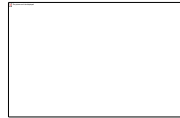
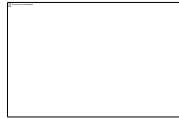

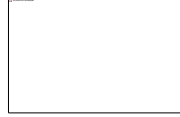
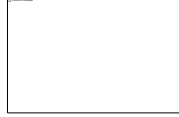







Contact: Mike Kvasnik (Fitzco, Inc.) at 1-800-367-8760.

Drierite: 5lbs Indicator Mesh #8

ZipLoc™ plastic bags (food storage size), 1 per participant

Airtight plastic container for storage of ZipLoc™ bags













APPENDIX 2 Sample Label Sheet (Bar Codes)

			Place this end on vial first 	Place this end on vial first 	Place this end on vial first 
CC0001 Draw Tube 1 Serum (Red) 10 mL	CC0001 Draw Tube 2 EDTA 10 mL	CC0001 Draw Tube 3 Serum (Red) 10 mL	CC0001 Vial # 01 Green Cap Serum	CC0001 Vial # 02 Green Cap Serum	CC0001 Vial # 03 Green Cap Serum
			Place this end on vial first 	Place this end on vial first 	Place this end on vial first 
CC0001 Draw Tube 4 Serum (Red) 10 mL	CC0001 Draw Tube 5 Serum (Red) 10 mL	CC0001 Draw Tube 6 Serum (Red) 10 mL	CC0001 Vial # 04 Green Cap Serum	CC0001 Vial # 05 Green Cap Serum	CC0001 Vial # 06 Purple Cap Serum
			Place this end on vial first 	Place this end on vial first 	Place this end on vial first 
CC0001  Urine Cup 3 oz		CC0001  Blood blotter	CC0001 Vial # 07 Purple Cap Serum	CC0001 Vial # 08 Purple Cap Serum	CC0001 Vial # 09 Purple Cap Serum
		Place this end on vial first 	Place this end on vial first 	Place this end on vial first 	Place this end on vial first 
CC0001  Toe Nail	CC0001  Finger Nail	CC0001 Laboratory Processing Form	CC0001 Vial # 10 Purple Cap Serum	CC0001 Vial # 11 Orange Cap Whole Blood	CC0001 Vial # 12 Orange Cap Whole Blood














**APPENDIX 2 Sample Label Sheet (Bar Codes)**

Place this end on vial first  CC0001 Vial # 13 Brown Cap Serum	Place this end on vial first  CC0001 Vial # 14 Brown Cap Serum	Place this end on vial first  CC0001 Vial # 15 Clear Cap Serum
Place this end on vial first  CC0001 Vial # 16 Clear Cap Serum	Place this end on vial first  CC0001 Vial # 17 Clear Cap Serum	Place this end on vial first  CC0001 Vial # 18 Clear Cap Serum
Place this end on vial first  CC0001 Vial # 19 Clear Cap Serum	Place this end on vial first  CC0001 Vial # 20 Clear Cap Serum	Place this end on vial first  CC0001 Vial # 21 Clear Cap Serum
Place this end on vial first  CC0001 Vial # 22 Clear Cap Serum	Place this end on vial first  CC0001 Vial # 23 Clear Cap Serum	Place this end on vial first  CC0001 Vial # 24 Clear Cap Serum

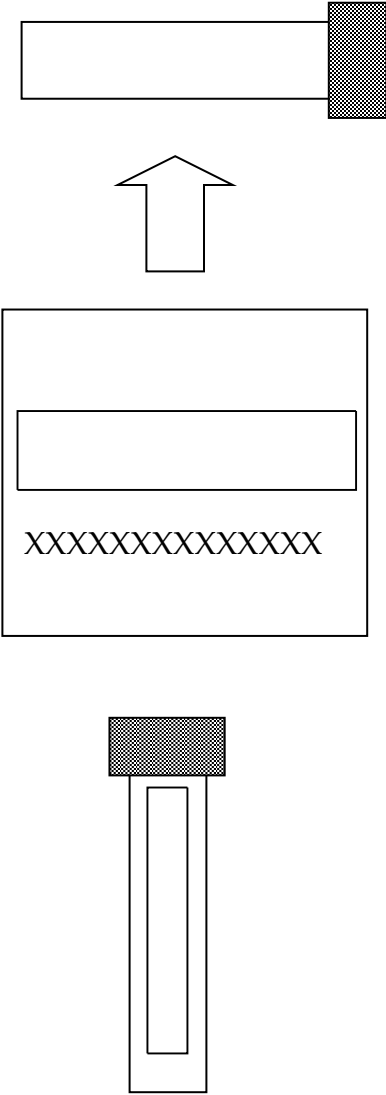
**APPENDIX 2 Sample Label Sheet (Bar Codes)**

<p>Place this end on vial first</p>  <p>CC0001 Vial # 25 Clear Cap Serum</p>	<p>Place this end on vial first</p>  <p>CC0001 Vial # 26 Clear Cap Serum</p>	<p>Place this end on vial first</p>  <p>CC0001 Vial # 27 Yellow Cap Urine</p>
<p>Place this end on vial first</p>  <p>CC0001 Vial # 28 Yellow Cap Urine</p>	<p>Place this end on vial first</p>  <p>CC0001 Vial # 29 Yellow Cap Urine</p>	<p>Place this end on vial first</p>  <p>CC0001 Vial # 30 Yellow Cap Urine</p>
<p>Place this end on vial first</p>  <p>CC0001 Vial # 31 Yellow Cap Urine</p>	<p>Place this end on vial first</p>  <p>CC0001 Vial # 32 Yellow Cap Urine</p>	<p>Place this end on vial first</p>  <p>CC0001 Vial # 33 Yellow Cap Urine</p>

**APPENDIX 3 Sample Processing Checklist**

- Crushed ice in ice bucket or plastic tub
- Pipets: 1.0 mL volumes and pipet tips
- Labeled cryovials in rack
- Lab coat and gloves
- Biohazardous waste disposal
- Horizontal centrifuge
- Balance tubes for the centrifuge
- 10% bleach solution
- Styrofoam container for freezing cell cryovials
- Freezer boxes with 9 x 9 grid
- Rubber bands
- Aluminum foil

### Label Orientation on Cryovial



**APPENDIX 6 Laboratory Forms**

These are not available electronically.