Contents

SOP 02: P-MRI Pre-DRE Urine DRE Procedure and Post-DRE Urine Collection (required), Processing, Shipping and Storage

1.0 Urine SOP Overview

Sites are being asked to collect 30-60 mL+ of pre-DRE urine and 30-60 mL+ of post-DRE urine into 100 mL cup.

- If collecting ALL specimens (Pre-DRE Blood/Urine and Post-DRE Urine) on the same day, they must be collected in the following order: Pre-DRE blood, Pre-DRE urine, (perform DRE), then collect Post-DRE urine.
- All samples must be collected prior to any procedures (such as biopsy) and prior to any anesthesia being administered.
- If Pre and Post DRE specimens are procured on different days, the order for blood and urine collection does not matter.
- Do not collect urine (or blood) from a patient who has evidence of urinary tract infection (treated or untreated) within the past 2 days.
- Do not collect urine (or blood) from a patient who has had a cystoscopy or catheter within the past 2 days.
- Each collected *30-60 mL+ of urine (pre and post-DRE) will be processed to produce the following specimens:
  - Two (2) **raw urine** at 1.0 mL, in 2 mL tubes with blue caps
  - Four (4) **whole urine** at 2.5 mL, in 5 mL tubes with clear caps, PLUS 2.5mL of Urine Transport Medium/buffer.
  - One (1) **urine sediment/pellet** at 50 µL, in 1.5 mL tube with flip cap
  - Four (4) **urine supernatant** at 5 mL, in 5 mL tubes with clear caps
  - *If less than 30 mL is collected in the cup, proceed with the “Minimum Processing Plan…” in section 3.3.
- Any remaining urine can be kept locally or discarded.

2.0 Urine Collection

100 mL collection cups are provided by the University of Michigan. If collecting pre and post-DRE urine on the same day, please clearly identify the cups prior to collection so they don’t get mixed up during transport and processing.

A sterile technique is not required for the procurement of the urine specimens.

Before the biopsy and prior to any anesthesia a pre-DRE urine specimen will be collected as follows:

Step 1. Provide the subject with a 100mL urine collection cup. Men should be instructed to fill the urine cup starting with the first drop of urine until the cup is full or their bladder is empty. If the subject is unable to provide this quantity, collect at least 10 mL and proceed with the “Minimum Processing Plan…” in Section 3.3

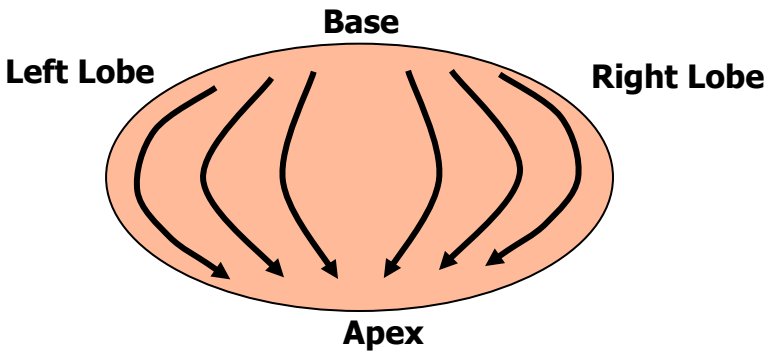
Step 1. Label the collection cup with provided “Kit # Pre-DRE Urine Cup” label and record the collection information on the Pre-DRE specimen worksheet.

Step 2. If sample is not being immediately processed, store on ice or at 4°C, for no longer than four hours, until processing begins.

2.1 Attentive DRE Procedure:

The clinician will perform a standard of care DRE as indicated below and document any suspicious nodules or other abnormalities (Figure 1). Apply firm pressure on the prostate from the base to the apex and from the lateral to the median line of each lobe as shown below. Apply enough pressure to slightly depress the prostate surface. Perform exactly three strokes per lobe.

Figure 1: Attentive Digital Rectal Exam of the Prostate



2.2 Post-DRE Urine Collection

Before the biopsy and prior to any anesthesia a pre-DRE urine specimen will be collected as follows:

Step 2. Provide the subject with a 100 mL urine collection cup. Men should be instructed to fill the urine cup starting with the first drop of urine until the cup is full or their bladder is empty. If the subject is unable to provide this quantity, collect at least 10 mL and proceed with the "Minimum Processing Plan…" in Section 3.3

Step 3. Label the collection cup with provided "Kit # Post-DRE Urine Cup" label and record the collection information on the yellow Post-DRE specimen worksheet.

Step 4. If sample is not being immediately processed, store on ice or at 4°C, for no longer than four hours, until processing begins.

3.0 Urine Processing (same for Pre or Post-DRE) – both required

Maintain specimen container on ice and transport to laboratory for processing. Urine processing should begin within 4 hours of collection.

Pre and post-DRE samples must be treated as different specimen types. If processing pre and post-DRE samples at the same time, **do not use the same pipette** tip or transfer pipette to process the 2 kinds of specimens.

If processing pre and post-DRE samples at the same time, keep the samples differentiated by labeling the aliquots prior to filling. Note that the post-DRE specimen worksheet is printed on yellow paper.

3.1 Supplies:

Sites are responsible for providing the following supplies:

- Freezer boxes "5x5x2" (with 9x9 inserts included), Fischer part #03-395-464
- Freezer boxes "5x5x4" (with 7x7 insert included), Sarstedt part # 95.064.949

For a list of provided supplies and materials: needed, see *SOP 05: Supplies and Kit Making*

3.2 Urine Processing Instructions when 30-60 mL+ is collected (if less than 30 mL is collected, see section 3.3: Minimum Processing Plan…)

3.2.1 Raw Urine

Step 1. Using the transfer pipette, transfer 5 mL of the remaining **raw urine** each into two (2) 5 mL tubes with blue caps.

Step 2. Label the tubes with labels RU1-RU2 from the appropriate kit (if not already labeled).

Step 3. Place the aliquot in a 9x9 gridded box (which should already be labeled according to the Manual of Operations: Section 14.5/Appendix 5), then place the box in a –70^{0}C or colder freezer until shipping to FNLCR.

Step 4. Complete the raw urine section of the worksheet from the kit.

3.2.2 Whole Urine Tube

Step 1. To resuspend cells in the sample, invert the initial voided urine sample (in urine collection cup) 5 times (do not shake as it will cause foam and bubbles).

Step 2. Prepare four **whole urine** tubes by placing 1.5 mL of buffer/Urine Transport Medium plus 3.5mL of urine from the collection cup in each tube. Total volume will be 5.0 mL, see Figure 2. If less than 3.5mL of urine is available, combine equal parts buffer and urine.

Figure 2: Whole Urine Tube

Step 3. Screw the cap on the transfer tube tightly, then invert each transport tube 5 times to mix.

Step 4. Label the tubes with labels WU1-WU4 from the appropriate kit (if not already labeled).

Step 5. Place the aliquot in a 7x7 gridded tall box (which should already be labeled according to the Manual of Operations: Section 14.5/Appendix 5), then place the box in a –70^{0}C or colder freezer until shipping to FNLCR.

Step 6. Complete the whole urine section of the worksheet from the kit.

3.2.3 Urine Cell Pellet/Sediment

Step 1. Centrifuge the remaining urine at 1000g for 10 minutes at 4°C **with brake OFF** being the preferred method. The method must be documented on the worksheet for tracking in VSIMS specimen tracking system.

Step 2. Transfer the supernatant to a 50 mL conical tube labeled with a kit # for processing of supernatant aliquots (Section 3.2.4), leaving 50-100 µL behind.

Step 3. Use the small remaining volume to resuspend the **urine cell pellet** and transfer 50-100 µL to a 1.5 mL tube, with a flip cap.

Step 4. Label the tube with the USE1 label from the appropriate kit (if not already labeled).

Step 5. Place the aliquot in a 9x9 gridded box (which should already be labeled according to the Manual of Operations: Section 14.5/Appendix 5), then place the box in a –70^{0}C or colder freezer until shipping to FNLCR.

Step 6. Complete the urine sediment section of the worksheet from the kit.

3.2.4 Urine Supernatant

Step 1. Aliquot 5 mL of **urine supernatant** each into four (4) 5 mL tubes with clear caps.

Step 2. Label the tubes with labels US1-US4 from the appropriate kit (if not already labeled).

Step 3. Place the aliquot in 7x7 gridded tall box (which should already be labeled according to the Manual of Operations: Section 14.5/Appendix 5), then place the box in a –70^{0}C or colder freezer until shipping to FNLCR.

Step 4. Complete the urine supernatant section of the worksheet from the kit.

3.3 *Minimum Processing Plan when <u>LESS THAN 30 mL of</u> Urine Collected

If less than 30 mL of urine is collected, refer to individual processing procedures in Section 3.2 and prepare specimens in the following order as the sample volume allows:

**2 whole urine tubes at 2.5 mL urine plus 2.5mL buffer. **Absolute minimum required for eligibility.

1 raw urine at 1 mL

1 urine cell pellet at 50 µL

1-4 urine supernatant at 5 mL, depending on volume, create as many additional aliquots as possible

4.0 Urine Processing Flow Chart

Prostate-MRI Pre (Required) and Post-DRE (Required) Urine Processing Flow Chart

V6.0: 03/16/2024

Original collection container
100 mL cup – 30-60 mL urine

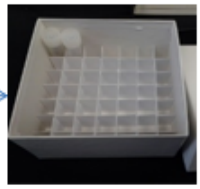
Maintain on ice, process within 4 hours of collection. Gently invert the collection cup five times to re-suspend the specimen

***If less than 30 mL of urine is collected** proceed to "Minimum Processing Plan"

1

Raw urine: 2 x 5 mL tubes

Aliquot 5 mL into each tube

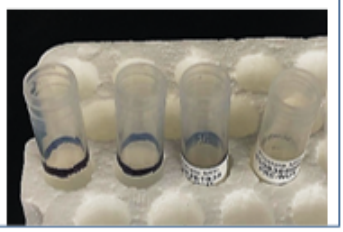


7x7 tall box, store at -70°C or colder within 4 hours of collection

2

Whole Urine: 4 x 5 ml tubes

Aliquot 3.5 mL of Urine Transport Medium/buffer plus 1.5 mL of urine into each tube. Invert 5 times to mix.

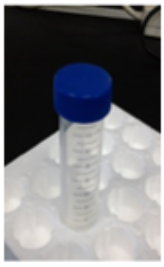


7x7, tall box, store at -70° C or colder within 4 hours of collection

3

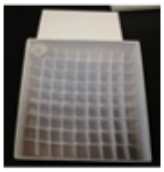
Centrifuge
Transfer remaining urine to 50 mL conical tube, centrifuge 10 min at 1000g at 4°C

Transfer supernatant to new 50 mL conical tube, leaving behind 50-100 µL



4

Pellet (sediment): one 1.5 mL tube
Use the small remaining volume to re-suspend the urine c… pellet and transfer it (50 … one labeled 1.5 mL tube



9x9 box, store at -70° C or colder within 4 hours of collection

5

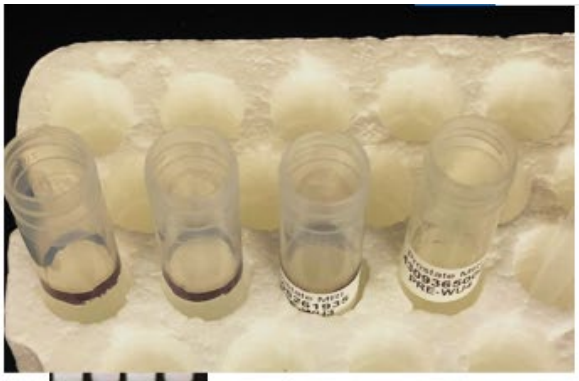
Supernatant: 2 x 5 mL tubes
Transfer 5 mL of supernatant into 2, 5 mL tubes.

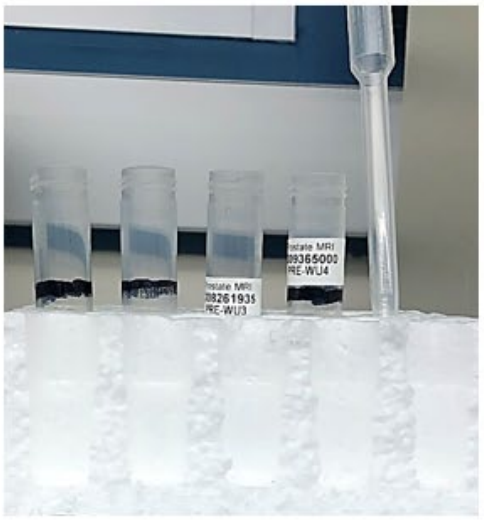
7x7, tall box, store at -70° C or colder within 4 hours of collection

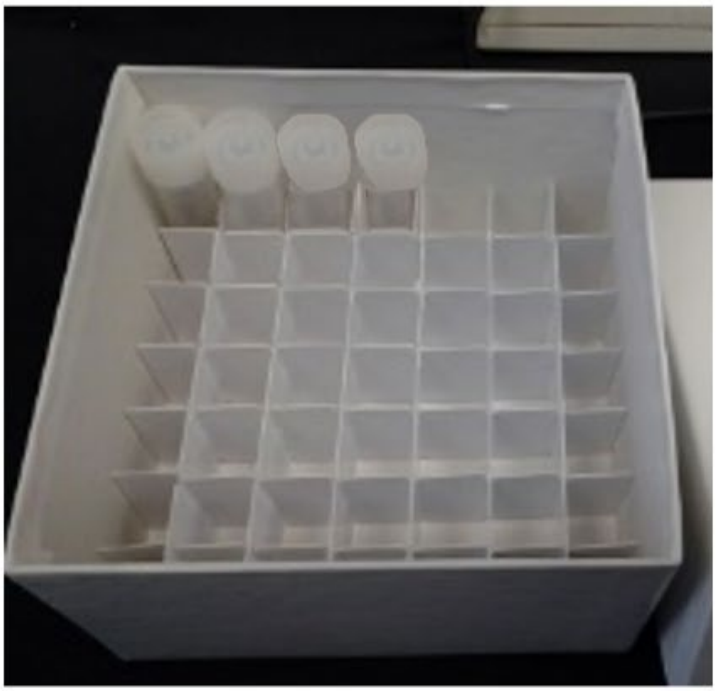
All specimens:
Label specimens with provided ID labels, complete specimen worksheets, enter information into VSIMS

***Minimum Processing Plan:** Create the following specimens, in this order as the volume of the actual sample produced allows (using the individual specimen processing instructions above).

- **5 mL of whole urine (divided into 2 specimens of 5 mL whole urine, plus 1.5 mL of Urine Transport Medium/buffer) for a total of 3.5 mL specimens (whole urine). **Absolute Minimum!
- 2 raw urine at 5 mL
- 1 urine cell pellet at 50 µL
- 1-4 urine supernatant at 5 mL, depending on volume, create as many additional aliquots as possible

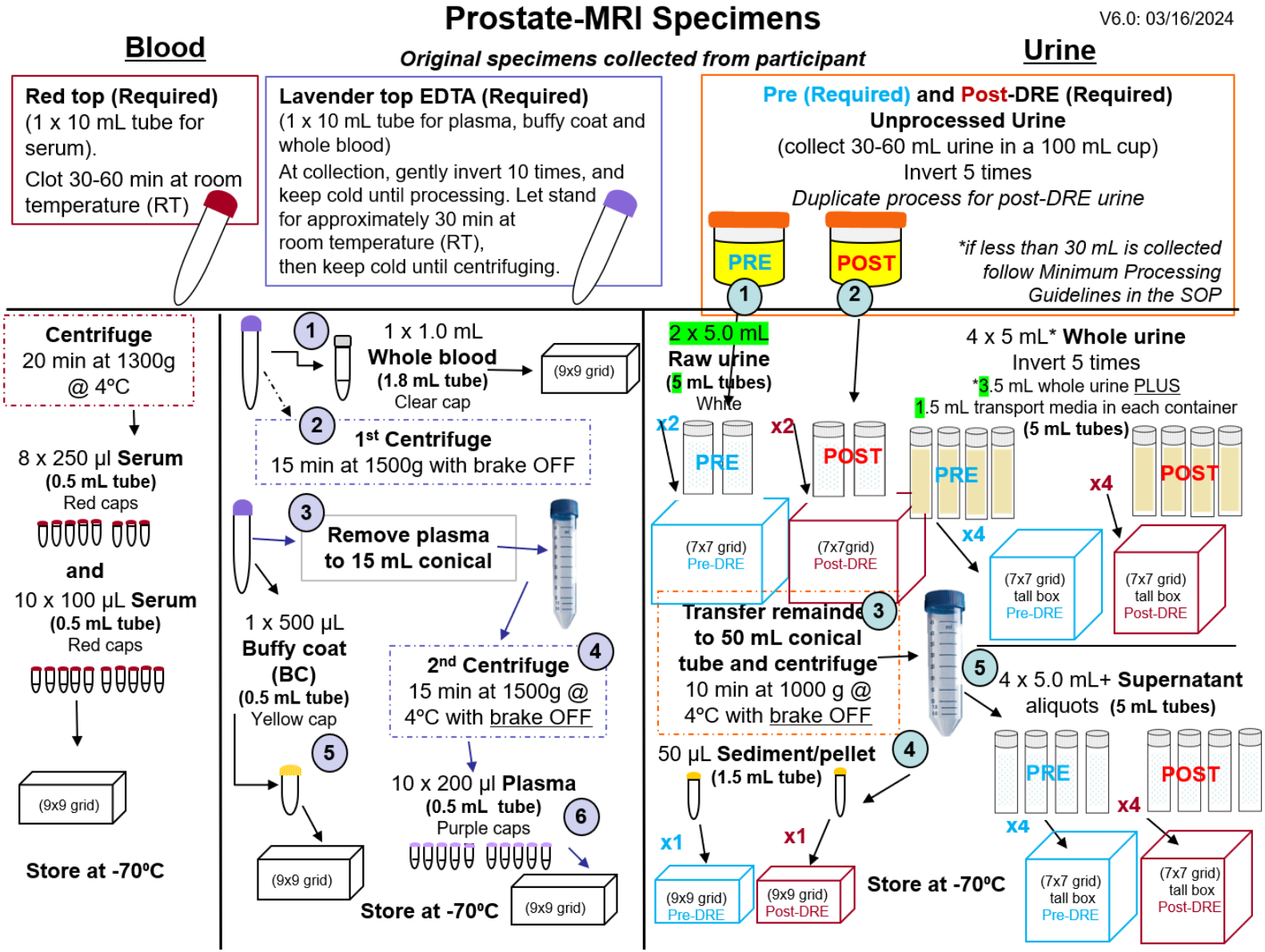






2

5.0 PMRI Specimen Plan



6.0 Shipping Overview

Specimens which have been entered into the VSIMS database as part of the Prostate MRI Study (P-MRI) will be shipped to the Frederick National Laboratory for Cancer Research (FNLCR) in Frederick, MD (aka NCI-Frederick) for inventory, blinding, array, and shipment to testing laboratories. Post-analysis specimen remnants will return to the FNLCR for long-term storage at the NCI at Frederick Central Repository (repository).

6.1 Preparing for Shipment

Step 1. Plan to ship only Monday – Wednesday

Step 2. Assess which boxes of specimens will be shipped and complete the following steps In VSIMS >Specimen Tracking:

a) Check the Missing Processing Qs Report for a list of specimens that are missing answers to the processing questions, see MOP section 5.6.3

b) Check the Box Space Report to ensure the number of specimens in each box is correct. This eliminates undocumented samples from being shipped, see MOP section 5.6.4

c) Prepare a list of Boxes to be shipped (by Box Number) and review the Box Maps. Perform spot checks on several boxes by pulling a sample from the box and making sure it's location in the physical box matches the Box Map from VSIMS.

Step 3. The shipping site must purchase their own shipping container that will adequately hold the intended number of specimen boxes to shipped, plus the necessary amount of dry ice to keep the samples frozen during shipment and passes the IATA drop test from a height of not less than 1.2 m.

Step 4. Obtain a UPS shipping label by contacting Javed Siddiqui siddiqui@med.umich.edu at the University of Michigan. Provide the planned date of shipment, the box dimensions and the estimated weight (including dry ice).

Step 5. Contact the "receiving lab" Frederick National Laboratory for Cancer Research (FNLCR) to confirm they will be able to receive the package on the intended delivery date by emailing: NCI-FrederickCSPBPTLStaff@mail.nih.gov (this address is monitored by multiple staff members). Sites should only ship Monday through Wednesday. However, the FNLCR is closed on Federal holidays and may also have abbreviated ship windows for the weeks of Thanksgiving and Christmas. Also, be aware of email notifications from the DMCC regarding FNLCR closures during holidays and inclement weather.

Step 6. The site must supply the necessary dry ice needed for shipping. If needed, order ahead, but plan to use minimum of 55 pounds (25kg) of dry ice or each box. Use more for larger boxes, proportional to the size of the box.

6.2 "Shipping" Specimen Boxes in VSIMS: www.compass.fhcrc.org/vsims

1. To "ship the specimens in VSIMS electronically", you must have a list of the Box ID Numbers (assigned during original key entry) to be shipped and the UPS tracking number to be used (obtained on the shipping label provide by Javed). For complete instructions please review section 5.8 of the MOP.

2. Please take time to perform the following audit checks prior to sealing up the box for shipment. This can be done in advance in the lab during preparation so there are not any "surprises" on shipping day.
    a. Spot-check a few of the samples. Pull a couple of samples from the box and check to see if they are on the shipping list.
        i. If they are not on the shipping, the wrong box may have been pulled from the freezer.
        ii. Carefully inventory the box DO NOT SHIP SAMPLES THAT ARE NOT ON THE MANIFEST. If time is of the essence, pull the samples from the box, ensure they are labeled correctly and set them aside (keeping them frozen on dry ice) for later review and shipping at a later time.
    b. If necessary verify box contents with the use of a "box map" (in addition to the shipping list).

	1	2	3	4	5	6	7	8	9
A	1	2	3	4	5	6	7	8	9
B	10	11	12	13	14	15	16	17	18
C	19	20	21	22	23	24	25	26	27
D	28	29	30	31	32	33	34	35	36
E	37	38	39	40	41	42	43	44	45
F	46	47	48	49	50	51	52	53	54
G	55	56	57	58	59	60	61	62	63
H	64	65	66	67	68	69	70	71	72
I	73	74	75	76	77	78	79	80	81

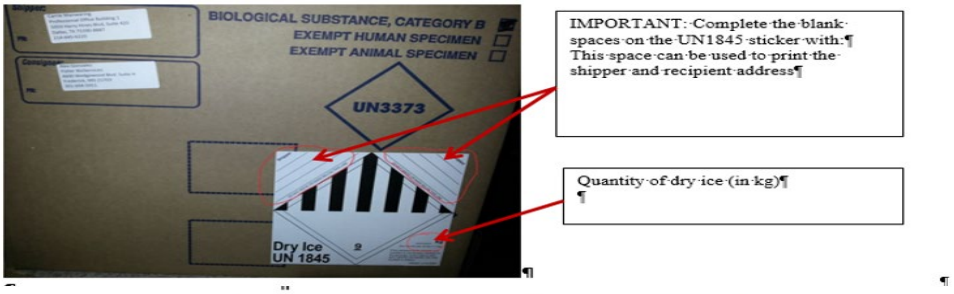
    c. Do not "ship the samples in VSIMS" until the number of samples on the shipping list matches the number of samples in the box. Enter the shipping date and tracking number for courier in VSIMS.
3. When specimens are successfully "shipped in VSIMS" a shipping manifest is created in VSIMS and an automated e-mail notification is sent to the FNLCR to notify them of an incoming along with the shipping manifest and courier tracking number. The shipping site should print 2 copies of the shipment manifest. Place one in the box being shipped and keep the other with the study documentation.

6.3 Packing Frozen Samples for Shipment

1. Pack the samples according to Standard UN 3373 (IATA Shipping Instructions 650) for "Biological Substance, Category B", i.e. triple packaging with two water tight and pressure safe layers with absorbent material in between. Triple packaging consists of the following:
    a. A leak proof primary receptacle (Eg. Cryovial)
    b. A leak proof secondary packaging (Eg. biohazard bag + 95kPa sleeve; or 95kPa Biohazard bag)
    c. An outer rigid packaging of adequate strength for its capacity, mass and intended use (Eg. cardboard shipping box with inner foam box)
2. For liquids, absorbent material in sufficient quantity to absorb the entire contents must be placed between the primary receptacle(s) and the secondary packaging.
3. Marking Requirements: Packages containing UN3373 materials must be clearly marked with the proper shipping name of "Biological substance, Category B" with the characters being at least 6 mm high. Packages must also have the mark illustrated in Packing Instruction 650 clearly and legibly displayed on the external surface of the outer packaging adjacent to the proper shipping name. The UN3373 mark must be in a square on point configuration (diamond shaped) with each side being a minimum of 50 mm (or 2 inches) in length with the UN3373 characters being at least 6 mm in

height. In addition, UN1845 stickers will be use in accordance with IATA regulations for shipping dry ice.

Biological substance, Category B



4. Include sufficient dry ice for the planned shipping time and include enough dry ice to protect the samples in the event of a one-day delay of transit. Note: Please do not try to save shipping costs by putting less dry ice in the package. Thawed samples cannot be used for this research.
5. The site must ensure that the container is large enough to hold sufficient dry ice to ensure samples arrive frozen. Dry Ice: Use minimum 55 pounds (25kg) for each box. Use more for larger boxes, proportional to the size of the box.
6. Place the box for scheduled pick up on the same day. Or call to schedule a pick up for the same day. Please check to make sure the box was picked up and that samples are not left sitting out. If something goes wrong, please resupply the dry ice before rescheduling another pick up.

6.4 Specimen Shipping Address & Contact Information

All specimens must be shipped via Priority Overnight for delivery the next day. **Only ship on only Monday, Tuesday or Wednesday** to avoid shipments arriving on holidays or weekends.

The shipping label placed on the container (from Javed Siddiqui, see section 7.1, step 4, above) should have the following address:

Norma Diaz
BioProcessing Laboratory
4600 Wedgewood Blvd
Suite K
Frederick, MD 21703
(301) 732-8252
NCI-FrederickCSPBPTLStaff@mail.nih.gov