

SOP 01: P-MRI Pre-DRE BLOOD SPECIMEN COLLECTION, PROCESSING, SHIPPING & STORAGE

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1.0 Blood SOP Overview

Sites are being asked to collect 20 mL of blood.

- ❖ 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 blood (or urine) from a patient who has evidence of urinary tract infection (treated or untreated) within the past 2 days.
- ❖ Do not collect blood (or urine) from a patient who has had a cystoscopy or catheter within the past 2 days.

The collected 20 mL of blood will be processed to produce the following blood specimens:

- **10 mL of blood in Red Top Tube, yielding:**
 - Eight (8) **serum** aliquots at 250 µL stored in 0.5 mL microfuge tubes
 - Ten (10) **serum** aliquots at 100 µL, stored in 0.5 mL microfuge tubes
 - If a site is unable to provide eighteen aliquots, please send all that is obtained
- **10 mL of blood in Lavender EDTA plasma tube, yielding:**
 - One (1) **whole blood** aliquot prior to centrifugation, for DNA isolation: at 1 mL in a 1.8 mL conical tube with a clear cap
 - Ten (10) **plasma** aliquots at 200 µL, stored in 0.5 mL microfuge tubes with purple caps
 - If a site is unable to provide ten aliquots, please send what is obtained
 - One (1) **buffy coat** aliquot at 500 µL, stored in 0.5mL tube, with yellow cap

Any remaining serum, plasma or buffy coat can be kept locally or discarded.

2.0 Blood Collection

Objective: To collect biologic samples on men prior to biopsy, in association with clinical information and common data elements appropriate for evaluation of prostate cancer.

Go to <http://www.bd.com/en-us/offerings/capabilities/specimen-collection/specimen-collection-resource-library?productline=3560&search=product+insert> and download instructions for use.

2.1 Supplies:

Sites are responsible for providing the following phlebotomy materials: Gloves, sharps Container, 21- gauge Butterfly needle, attached tubing and Luer adapter, Tourniquet, Antiseptic wipes, bandages, centrifuge as per SOP.

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

2.2 Procedure:

- Step 1.** Assemble the supplies to be used in obtaining the specimen. Do not label the vacutainer tubes until specimen is obtained.
- Step 2.** Put on disposable gloves.
- Step 3.** The patient should be comfortably seated in a venipuncture chair. The arm should be positioned on a slanting armrest in a straight line from the shoulder to the wrist. The arm should not be bent at the elbow.
- Step 4.** Apply a tourniquet 2 inches above the antecubital fossa or above area to be drawn with enough pressure to provide adequate vein visibility. Have the patient form a fist. Select the site for venipuncture.

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- Step 5.** Clean the forearm of the patient with antiseptic wipe in a circular motion beginning at the insertion site. Allow the antiseptic to dry.
- Step 6.** Anchor the vein by placing the thumb 2 inches below the site and pulling the skin taut to prevent the vein from moving. The holding finger is placed below the site, not above, to prevent accidentally sticking the finger with the needle.
- Step 7.** Using the dominant hand, insert either the butterfly needle or straight needle. Push the evacuated tube onto Luer adapter.
- Step 8.** Release the tourniquet once blood flow is established.
- Step 9.** Carefully remove the tubes when full without dislodging the needle. The tube will automatically stop filling when the vacuum is gone leaving the tube approximately three-fourths full.
- Step 10.** Lightly place a sterile gauze pad over the venipuncture site. Gently remove the needle.
- Step 11.** Apply pressure to the site with sterile gauze. Apply bandage. Instruct the patient to leave the bandage on for at least 15 minutes.
- Step 12.** Dispose of the needle in a sharps container.
- Step 13.** Tubes with additives must be thoroughly mixed. Erroneous test results may be obtained when the blood is not thoroughly mixed with the additive.
- Step 14.** Label the tubes with Serum Tube and Plasma Tube labels for the appropriate kit #
- Step 15.** Remove gloves and wash hands.
- Step 16.** Record the collection information on the specimen worksheets.

3.0 Serum: Processing and Storage

Objective: Serum is obtained from whole blood collected in red top vacutainer tube with no additives or clot activators. Specimens should be processed within a 4 hour window. If located at an offsite location, it is acceptable to preserve specimens on ice or at 4°C refrigerator until processing. A delay in processing because of transportation will not exclude a participant from eligibility. Serum processing is consistent with <http://www.bd.com/vacutainer/pdfs/VDP40163.pdf>

3.1 Supplies:

Sites are responsible for providing the following: Standard freezer boxes 5x5x2" (9x9 inserts included), Fischer part #03-395-464.

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

3.2 Procedure: Follow Universal Precautions and general safety practices while interacting with samples.

- Step 1.** Allow the blood specimen to clot for minimum of 30 minutes and maximum of 60 minutes at room temperature. If not immediately processed, store at 4°C for up to 4 hours (this should be documented on the specimen worksheet from the kit).
- Step 2.** Centrifuge for 20 minutes at 1300g-force, 4°C/39°F. If centrifuge uses RPM (revolutions per minute), see centrifuge product brochure for conversion.
- Step 3.** Using disposable bulb pipette, transfer serum into 0.5 mL tubes w/red caps: eight (8) at 250 µL and ten (10) at 100 µL.
- Step 4.** Label all aliquots with labels SE1-SE18 from the appropriate kit (if aliquots are not already labeled).
- Step 5.** Place 18 tubes 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°C or colder freezer until shipping to FNLCR.
- Step 6.** Complete the Serum Worksheet from the kit. Hemolyzed serum samples are to be noted as such.

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4.0 Whole Blood, cellular fraction (Buffy Coat) and Plasma specimens

Objective: Whole blood, plasma and cellular fractions are being collected using a 10 mL EDTA containing tube. Plasma is obtained from whole blood collected in plastic vacutainer tubes containing EDTA from Becton-Dickinson. At collection tubes are inverted for mixing 10 times. Specimens should be processed within a 4 hour window. If located at an offsite location, it is acceptable to preserve specimens on ice or at 4°C refrigerator until processing. A delay in processing because of transportation will not exclude a participant from eligibility. Processing is consistent with <http://www.bd.com/vacutainer/pdfs/VDP40161.pdf>

4.1 Supplies:

Sites are responsible for providing the following: Freezer boxes 5x5x2" (9x9 inserts included), Fischer part #03-395-464

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

4.2 Processing Procedure:

Always follow Universal Precautions and general safety practices while interacting with samples.

Allow specimen to stand at room temperature for approximately 30 minutes prior to centrifugation. If the centrifuge used is capable of refrigeration, then this time may be shortened. If not immediately processed, store at 4°C for up to 4 hours (this should be documented on the specimen worksheet from the kit).

4.2.1 Whole Blood:

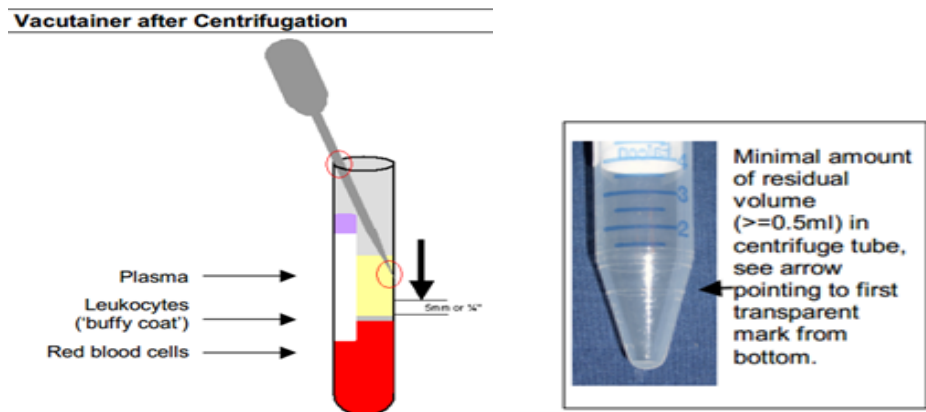
- Step 1.** Remove 1 mL of whole blood prior to centrifugation and store in one (1) 1.8 mL conical tube with a clear cap.
- Step 2.** Label the aliquot with the WB1 label from the appropriate kit (if not already labeled).
- Step 3.** Place the aliquot in a 9x9 gridded box (which should already be labeled according to the Manual of Operations: Section 14.5/Appendix 5), then place the box in a -70°C or colder freezer until shipping to FNLCR.
- Step 4.** Complete the whole blood section of the worksheet from the kit.

4.2.2 Buffy Coat:

- Step 1.** **With brake OFF**, centrifuge EDTA tube for 15 minutes at 1500g-force at 4°C/39°F. If centrifuge uses RPM (revolutions per minute), see centrifuge product brochure for conversion.
NOTE: As seen below, centrifugation separates plasma from white and red blood cells as shown on the figure above. Leaving sufficient residual volume in the tubes after the centrifugation and not disturbing the buffy coat when pipetting is the most critical step in the sample preparation process. DO NOT reduce plasma quality by reducing the residual volume when pipetting.
Buffy coat is visible as very small whitish band (see arrow below) above the red blood cells. Be careful not to disturb the buffy coat (cellular) layer in the original Vacutainer tubes. To minimize risk of aspirating cells from the buffy coat, hold Vacutainer upright and tilt the pipette to touch the Vacutainer in two positions (see red circles in the drawing) and slowly move pipette down while aspirating. Always place the pipette at the top of the plasma layer and STOP aspirating ≥ 5 mm or $\geq 1/4$ " above the buffy coat (cellular layer) in order to avoid contaminating the plasma with cells. If cells are aspirated, dispose pipette and Vacutainer. See figures 1 below.

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Figure 1: Buffy Coat



- Step 2.** When centrifuge has stopped, using a disposable bulb pipette, transfer the plasma layer to a 15 mL centrifuge tube labeled with a Kit# and with **the brake OFF**, centrifuge the 15 mL tube for 15 minutes at 1500g-force at 4°C/39°F (see section 4.2.3 for further plasma processing details).
- Step 3.** **Buffy coat:** From the collection tube, remove the buffy coat and transfer to one (1) 0.5 mL aliquot with a yellow cap.
- Step 4.** Label the aliquot with the BC1 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°C or colder freezer until shipping to FNLCR.
- Step 6.** Complete the buffy coat section of the worksheet from the kit.

4.2.3 Plasma:

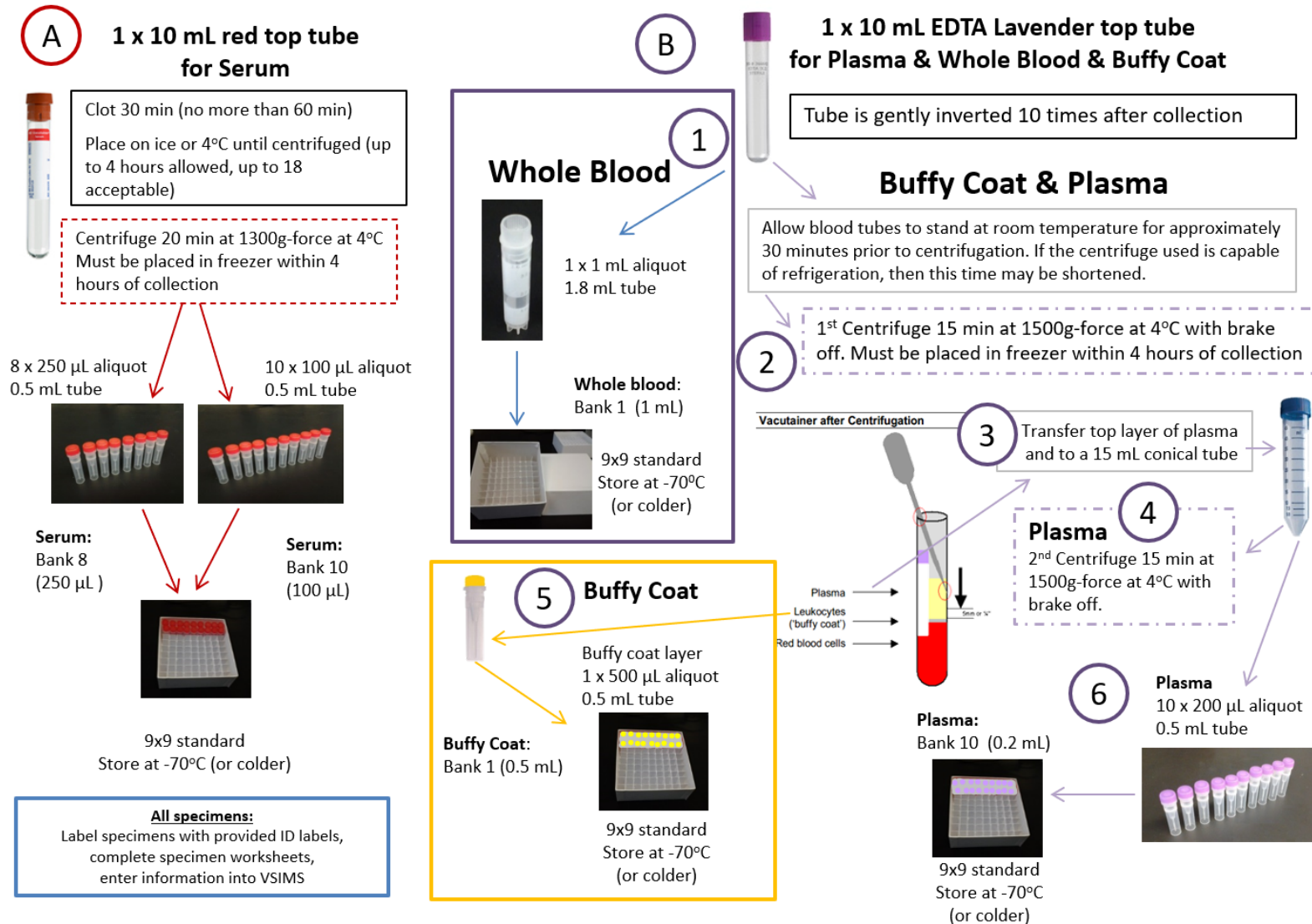
- Step 1.** When centrifuge has stopped, from the 15 mL centrifuge tube, aliquot 0.2 mL of plasma into ten (10) 0.5 mL tubes with purple caps.
- Step 2.** Label the aliquots with labels PL1-PL10 from the appropriate kit (if not already labeled).
- Step 3.** Place the aliquots in a 9x9 gridded box (which should already be labeled according to the Manual of Operations: Section 14.5/Appendix 5), then place the box in a -70°C or colder freezer until shipping to FNLCR.
- Step 4.** Complete the plasma section of the worksheet from the kit.

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5.0 P-MRI Blood Flow Chart

Prostate-MRI Blood Processing Flow Chart

V6.0: 03/16/2024



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6.0 PMRI Specimen Plan

Prostate-MRI Specimens

V6.0: 03/16/2024

Blood

Red top (Required)
(1 x 10 mL tube for serum).
Clot 30-60 min at room temperature (RT)



Lavender top EDTA (Required)
(1 x 10 mL tube for plasma, buffy coat and whole blood)
At collection, gently invert 10 times, and keep cold until processing. Let stand for approximately 30 min at room temperature (RT), then keep cold until centrifuging.



Original specimens collected from participant

Urine

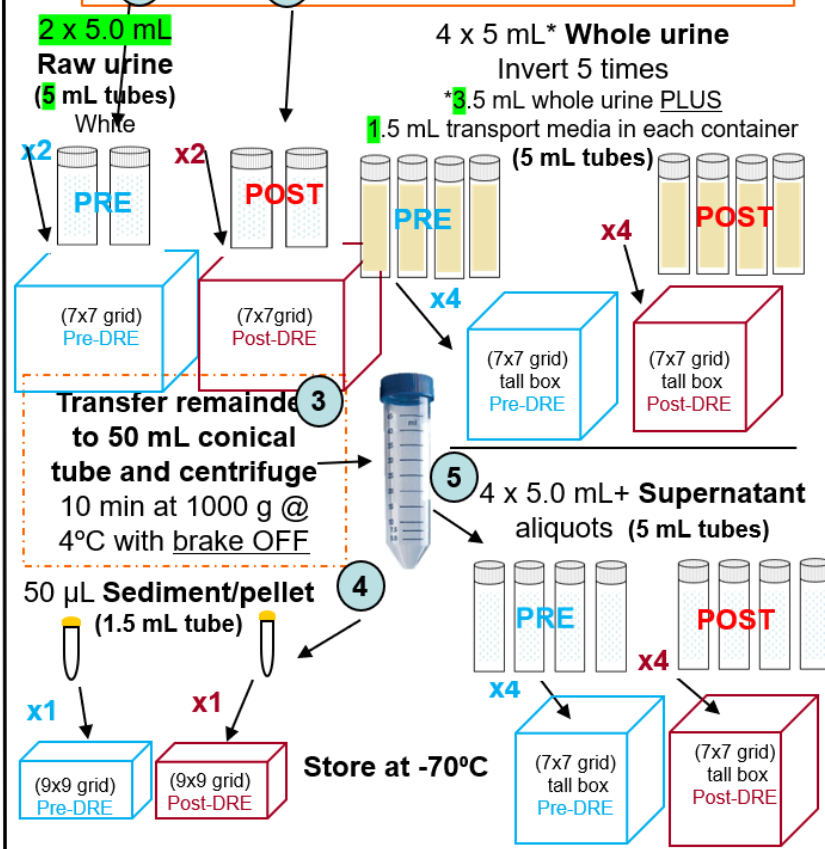
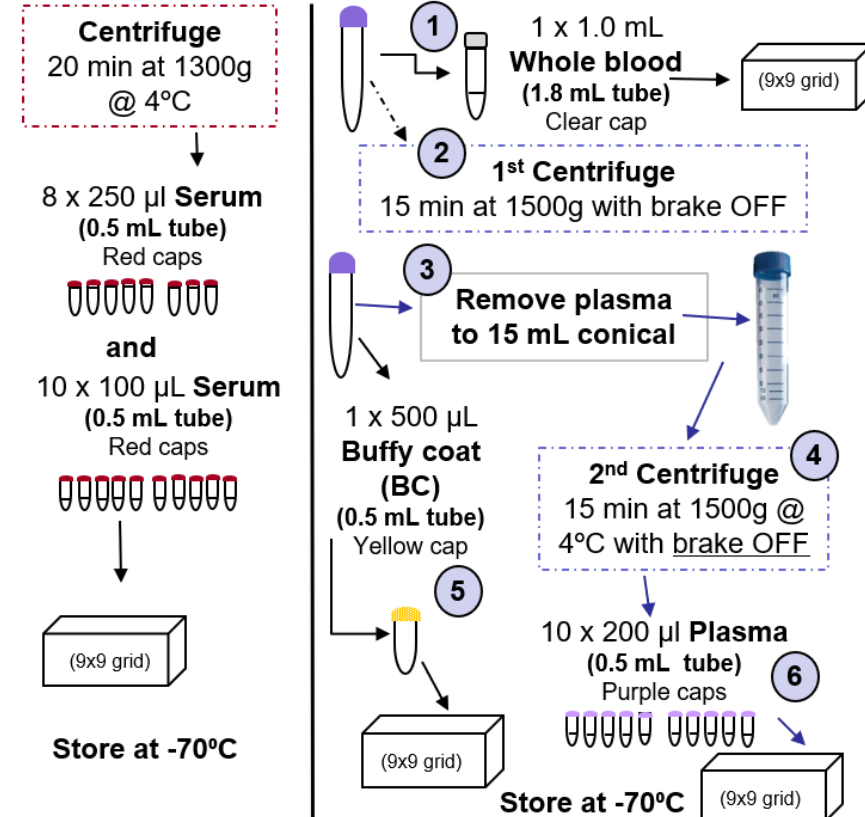
Pre (Required) and Post-DRE (Required)
Unprocessed Urine

(collect 30-60 mL urine in a 100 mL cup)
Invert 5 times

Duplicate process for post-DRE urine



*if less than 30 mL is collected follow Minimum Processing Guidelines in the SOP



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7.0 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).

7.1 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.

7.2 “Shipping” Specimen Boxes in VSIMS: www.compass.fhcr.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.

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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.

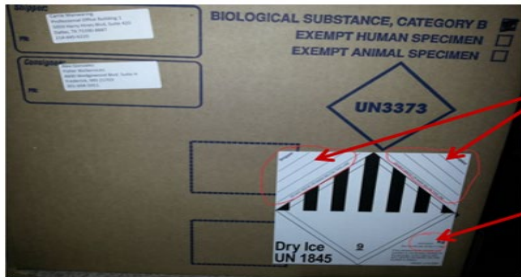
7.3 Packing Frozen Samples for Shipment

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.

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Biological substance, Category B



IMPORTANT: Complete the blank spaces on the UN1845 sticker with: This space can be used to print the shipper and recipient address

Quantity of dry ice (in kg)



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.

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7.4 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