National Liver Cancer Screening Trial (TRACER)
Biospecimen Standard Operation Procedure (SOP)

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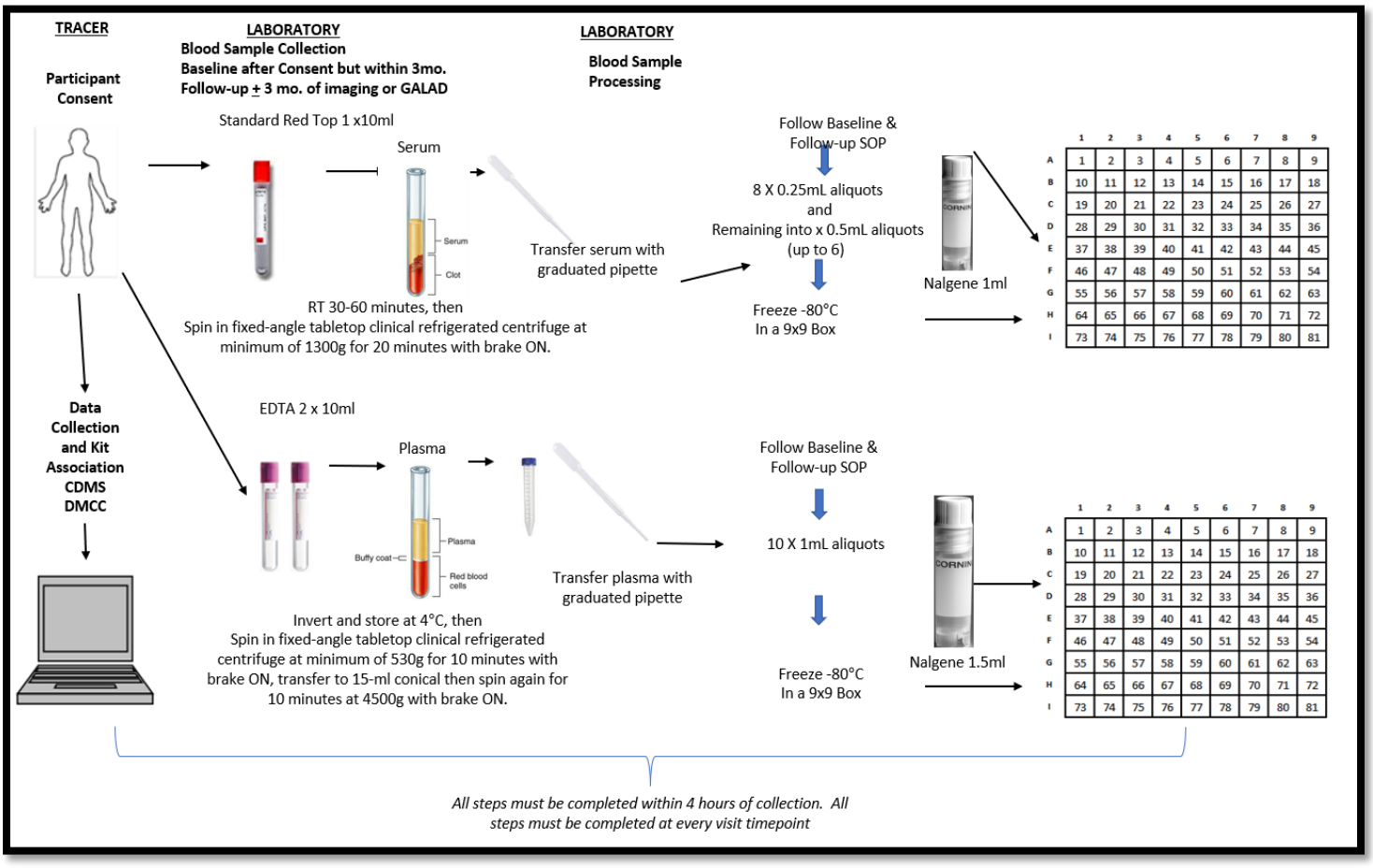
1. Blood Sample Collection and Processing Overview Diagram:

Figure 1



2. Collection Schedule

Table 1

Visit Name	Screening /Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11[A]
Month	0	6	12	18	24	30	36	42	48	54	60
Visit Window (months)	0	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3
Serum	X	X	X	X	X	X	X	X	X	X	X
Plasma	X	X	X	X	X	X	X	X	X	X	X

A=FU visits for HCC surveillance occur up to 10 years with visits every 6 months

3. Purpose:

The purpose of this procedure is to outline the process for collecting, processing, storing and shipping of blood specimens collected for the National Liver Cancer Screening Trial (TRACER).

A. Minimum equipment requirements for each site:

1. -80ºC Freezer with emergency backup generators.
2. Crushed ice for EDTA tubes if a delay in processing is anticipated.
3. Fixed-angle tabletop clinical centrifuge, refrigerated (4ºC), must reach at least 3000g (Brake On).
4. Microcentrifuge must reach at least 4500g (Brake On or deceleration set to 9).
5. Appropriate racks to hold tubes in upright position until processed.
6. Refrigerator for temporary sample storage prior to processing.

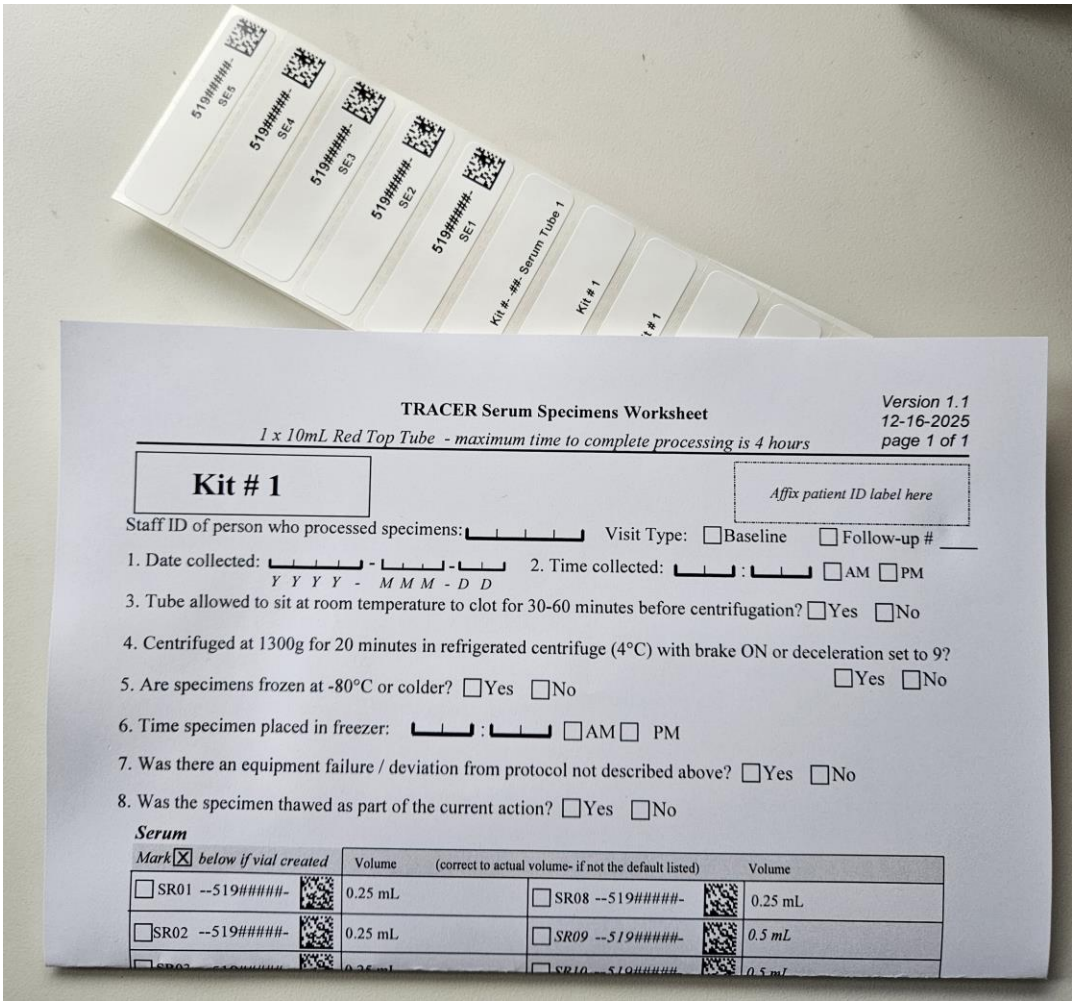
4. Collection Kits and Supplies:

Table 2 Collection and Processing Supplies	
Biospecimen Collection Kit must include:	
Provided by UTSW:	
a)	1 – 10mL Red top tubes (standard red top), for serum, BD Vacutainer #367820 
b)	2 – 10mL anticoagulant tube with EDTA (lavender top); K2EDTA Vacutainer tube BD #366643 
c)	Nalgene General Long-Term Storage Cryogenic Tubes, 1 mL, sterile, ThermoFisher Scientific Part #5000-1012 

d)	Nalgene General Long Term Storage Cryogenic tubes, 1.5ml, Sterile, Thermofisher Scientific part #5000-1020 (Note: Lavender inserts may also be provided, TBD) 
e)	Specimen ID labels and Collection and Processing Worksheets that are associated to a Kit# so the Specimen ID#s can easily be associated to the Patient ID in CDMS (printed by DMCC, sent to UTSW and included in Kit (**Figure 2**)
f)	Biohazard transport bag with absorbent tube sleeve
Purchased by Site:	
g)	15mL Falcon conical centrifuge tubes (Corning product#430766 from Fisher Scientific)
h)	Cardboard storage boxes (5” x 5” x 2”) (Fisher Catalog #11-678-24A)
i)	9x9 dividers for 2mL aliquot boxes (Fisher Catalog # 13-989-218)
j)	Plastic bulb transfer pipettes with 0.5mL and 1mL markings (Fisher Catalog #13-711-9CM)
k)	-80C freezer

5. Specimen Worksheets & Labels:

Figure 2-Worksheet and Label Set



TRACER Serum Specimens Worksheet

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1 x 10mL Red Top Tube - maximum time to complete processing is 4 hours

Kit # 1

Affix patient ID label here

Staff ID of person who processed specimens: _____ Visit Type: ☐Baseline ☐Follow-up # ____

1. Date collected: ____-____-____ (Y Y Y Y - M M M - D D) 2. Time collected: ____:____ ☐AM ☐PM
3. Tube allowed to sit at room temperature to clot for 30-60 minutes before centrifugation? ☐Yes ☐No
4. Centrifuged at 1300g for 20 minutes in refrigerated centrifuge (4°C) with brake ON or deceleration set to 9? ☐Yes ☐No
5. Are specimens frozen at -80°C or colder? ☐Yes ☐No
6. Time specimen placed in freezer: ____:____ ☐AM ☐ PM
7. Was there an equipment failure / deviation from protocol not described above? ☐Yes ☐No
8. Was the specimen thawed as part of the current action? ☐Yes ☐No

Serum

Mark ☒ below if vial created	Volume	(correct to actual volume- if not the default listed)	Volume
☐ SR01 --519#####-	0.25 mL	☐ SR08 --519#####-	0.25 mL
☐SR02 --519#####-	0.25 mL	☐ *SR09 --519#####-*	*0.5 mL*

Figure 3-Serum Worksheet

TRACER Serum Specimens Worksheet

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1 x 10mL Red Top Tube - maximum time to complete processing is 4 hours

Kit # -##-

Affix patient ID label here

Staff ID of person who processed specimens: ____ Visit Type: ☐Baseline ☐Follow-up # ____

1. Date collected: ____ - ____ - ____ (Y Y Y Y - M M M - D D) 2. Time collected: ____ : ____ ☐AM ☐PM

3. Tube allowed to sit at room temperature to clot for 30-60 minutes before centrifugation? ☐Yes ☐No

4. Centrifuged at 1300g for 20 minutes in refrigerated centrifuge (4°C) with brake ON or deceleration set to 9? ☐Yes ☐No

5. Are specimens frozen at -80°C or colder? ☐Yes ☐No

6. Time specimen placed in freezer: ____ : ____ ☐AM ☐PM

7. Was there an equipment failure / deviation from protocol not described above? ☐Yes ☐No

8. Was the specimen thawed as part of the current action? ☐Yes ☐No

Serum

Mark ☒ below if vial created	Volume (correct to … if volume is not the default listed)		Volume
☐ SR01 --519#####-	0.25 mL	☐ SR08 --519#####-	0.25 mL
☐ SR02 --519#####-	0.25 mL	☐ *SR09 --519#####-*	*0.5 mL*
☐ SR03 --519#####-	0.25 mL	☐ *SR10 --519#####-*	*0.5 mL*
☐ SR04 --519#####-	0.25 mL	☐ *SR11 --519#####-*	*0.5 mL*
☐ SR05 --519#####-	0.25 mL	☐ *SR12 --519#####-*	*0.5 mL*
☐ SR06 --519#####-	0.25 mL	☐ *SR13 --519#####-*	*0.5 mL*
☐ SR07 --519#####-	0.25 mL	☐ *SR14 --519#####-*	*0.5 mL*

Freezer #	9x9 Box #	row/column or slot … 1-9	Visually, the specimen appears... (circle all that apply)	SOP followed?	Status
			Hemolyzed: Appears pink-ish or red-ish in color **Lipemic:** Appears cloudy, turbid and/or opaque **Icteric:** Appears yellow-ish or green-ish in color	☐Yes ☐No ☐Unknown	☐ Normal ☐ Defective

If all tubes were not created, give reason: _____

_____

Comments: _____

_____

Figure 2-Plasma

TRACER Plasma Specimens Worksheet

2 x 10mL Purple Top Tube - maximum time to complete processing is 4 hours

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Kit # -##-

Affix patient ID label here

Staff ID of person who processed specimens: ____ Visit Type: ☐Baseline ☐Follow-up # ____

1. Date collected: ____ - ____ - ____ (Y Y Y Y - M M M - D D) 2. Time collected: ____ : ____ ☐AM ☐PM

3. Tube inverted 8-10 times? ☐Yes ☐No

4. Centrifuged at 530g for 10 minutes in refrigerated centrifuge (4°C) with brake ON? ☐Yes ☐No
If using a Sorvall ST40 or ST40R or similar centrifuge, spin for 10 min at 600g with deceleration set to 9, spin for 10min at 4500g with brake ON or deceleration set to 9, must use refrigerated centrifuge (4°C).

5. Was 2nd centrifuge at 4500g for 10 minutes in refrigerated centrifuge (4°C) with brake ON? ☐Yes ☐No*

*If No, what was the speed of the 2nd centrifuge_____

6. Are specimens frozen at -80°C or colder? ☐Yes ☐No

7. Time specimen placed in freezer: ____ : ____ ☐AM ☐PM

8. Was there an equipment failure / deviation from protocol not described above? ☐Yes ☐No

9. Was the specimen thawed as part of the current action? ☐Yes ☐No

Plasma

Mark ☒ below if vial created	Volume (correct to actual volume if not the default listed)		Volume
☐PL01 --519######-	1.0 mL	☐PL06 --519######-	1.0 mL
☐PL02 --519######-	1.0 mL	☐PL07 --519######-	1.0 mL
☐PL03 --519######-	1.0 mL	☐PL08 --519######-	1.0 mL
☐PL04 --519######-	1.0 mL	☐PL09 --519######-	1.0 mL
☐PL05 --519######-	1.0 mL	☐PL10 --519######-	1.0 mL

Freezer #	9x9 Box #	row/column or slot # 1-99	Visually, the specimen appears... (circle all that apply)	SOP followed?	Status
			Hemolyzed: Appears pink-ish or red-ish in color Lipemic: Appears cloudy, turbid and/or opaque Icteric: Appears yellow-ish or green-ish in color	☐Yes ☐No ☐Unknown	☐Normal ☐Defective

If all tubes were not created, give reason: _____

Comments: _____

Labeling the specimen aliquots:

All sites must label their specimens the same way in order to prevent future users of the specimen reference set from potentially identifying which site a specimen came from.

Labels should be affixed to the aliquot as follows: Affix the “left” side of the label first and wrap around the tube, counterclockwise. Some labels, when wrapped completely around, may obscure the first part of the ID number, but future label runs have a much smaller barcode so that nothing is obscured.

6. Blood Collection:

1. Do not collect blood specimens unless consent has been obtained.
2. Collect first blood after consent but within 3 mo. Collect longitudinal blood every 6 mo. ± 3 mo.
3. The study site staff must follow Universal Precautions as well as established internal guidelines for the collection of specimens and the handling and disposal of blood and specimen collection materials. All staff collecting and or shipping specimens must follow governmental laws for handling hazardous waste.
4. Gloves must be worn at all times when handling specimens. This includes during removal of the rubber stopper from the blood tubes, centrifugation, pipetting, disposal of contaminated tubes, and cleanup of any spills. Tubes, needles, and pipets must be properly disposed of in biohazard containers, in accordance with institutional requirements.
5. A total of 30 mL of blood will be collected. Order of blood draw collection should be:
    - 1 – 10mL Red Top Standard Tubes.
    - 2 – 10mL anticoagulant tube with EDTA (lavender top)—Note: These must be filled to line on label for proper EDTA blood ratios, invert gently according to manufacturer guidelines to mix.
6. After collection, label the collection tube with the DMCC provided Kit# label.
7. Complete both Specimen Collection Worksheets:
    - Make sure the pre-printed Kit ID number on the worksheet matches the Kit# labels.
    - Affix the correct DMCC provided Patient ID Label to the worksheets.
    - Complete the Date Collected and Time Collected.

7. Blood Processing:

A. Serum: Red Top Standard Serum Tube (1 x 10mL)

1. Blood will be collected in vacutainers with no anticoagulant (BD) in the form of red top tubes.
2. 10 mL of blood will be collected for serum at each visit time point.
3. Each sample must be processed and stored within 4 hours of collection.
4. After collection of the whole blood, allow the blood to clot by leaving it undisturbed standing upright at room temperature, do not invert. This usually takes at least 30 minutes, but not more than 60 minutes.
    a. After allowing the sample to clot, place the tube(s) in the refrigerator at 4°C until processing (if not processed immediately).
5. Spin for 20min at 1300g, must use refrigerated centrifuge (4°C) with brake ON or deceleration set to 9, depending on centrifuge model.
6. The resulting supernatant is the serum.
7. The samples should be maintained at 2-8°C using ice bucket while handling, *as needed.*
8. Aliquot a volume of 0.25 mL into 8 cryovials, and the remaining volume is to be aliquoted into 0.5mL aliquots, until all sample is depleted (up to 6 x 0.5 mL). **BE SURE TO AFFIX THE "SR" LABELS TO THE SERUM, NOT THE "PL", WHICH ARE FOR PLASMA)**
9. Store all aliquots at –80°C or colder.
10. Document the following data points of information on the Serum Worksheet.
    - Staff ID of person who processed specimens

- Visit type (Baseline or Follow-up #)
    1. Date of blood collection
    2. Time of blood collection
    3. Tube allowed to sit at room temperature to clot for 30-60 minutes before centrifugation
    4. Centrifuged at 1300g for 20 minutes in refrigerated centrifuge (4°C) with brake ON
    5. Confirm stored in -80°C freezer or colder
    6. Time placed in freezer
    7. Was there an equipment failure / deviation from protocol not described above
    8. Was specimen thawed as part of current action
- Number and volume of aliquots prepared
- If any freeze-thaw cycles occur with a sample for any reason, please add a comment

11. Label (or hand write with permanent marker) the box according to the diagram below then place the box in –80°C (or colder) freezer.
12. In CDMS associate the Kit# with the Patient ID and Time Point and complete the remaining questions on the Worksheet.
13. Track the location of Serum on the worksheet within box following **Figure 3**, specify the freezer, row & column in CDMS. Mark-up the freezer box as shown below before placing the box in your freezer.

B. PLASMA: EDTA Plasma tubes (2 x 10mL)

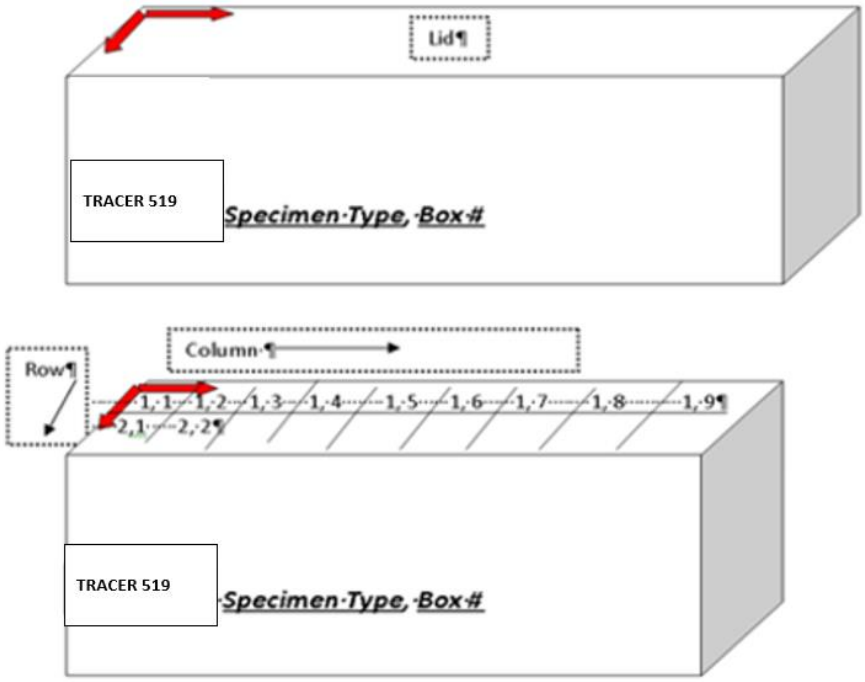
1. Collect whole blood into lavender top vacutainer with EDTA.
2. 20 mL of blood will be collected for plasma at each study visit.
3. Invert 8-10 times, store upright in 4°C refrigerator until processing.
4. Each sample must be processed and stored within 4 hours of collection.
5. Spin for 10min at 530g with break ON 9/9, must use refrigerated centrifuge (4°C). If using a Sorvall ST40 or ST40R or similar centrifuge, spin for 10 min at 600g with deceleration set to 9.
6. The resulting supernatant is plasma.
7. Immediately transfer the plasma into single new labeled 15-ml conical, avoid aspirating the buffy coat layer and the RBCs.
8. Spin for 10min at 4500g with break ON or deceleration set to 9, must use refrigerated centrifuge (4°C).
9. Document the actual speed used for 2nd centrifugation in CDMS specimen tracking system.
10. The samples should be maintained at 2-8°C using ice bucket while handling, *as needed.*
11. Aliquot a volume of 1mL into each cryovial. Continue to aliquot until all sample is used (up to 10 x 1mL) **BE SURE TO AFFIX THE “PL” LABELS TO THE PLASMA, NOT THE “SR”, WHICH ARE FOR SERUM)**.
12. Store all aliquots at –80°C or colder.
13. Document the following data point information on the sample acquisition form.
    - Staff ID of person who processed specimens
    - Visit type (Baseline or Follow-up #)

1. Date of blood collection
2. Time of blood collection
3. Tubes inverted 8-10 times
4. Centrifuged at 530g for 10 minutes in refrigerated centrifuge (4°C) with brake ON. If using a Sorvall ST40 or ST40R or similar centrifuge. Spin for 10 minutes at 600g with deceleration set to 9, spin for 10 min at 4500g with brake ON or deceleration set to 9, must use refrigerated centrifuge (4°C)
5. Was 2nd centrifuge at 4500g for 10 minutes in refrigerated centrifuge (4°C) with brake ON
    a) If not above, then at what speed was the second centrifuge
6. Are specimens frozen at -80°C or colder
7. Time placed in freezer
8. Was there an equipment failure / deviation from protocol not described above
9. Was specimen thawed as part of current action

- Number and volume of aliquots prepared
- If any freeze-thaw cycles occur with a sample for any reason, please add a comment

14. Label (or hand write with permanent marker) the box according to the diagram below then place the box in –80°C (or colder) freezer.
15. In CDMS associate the Kit# with the Patient ID and Time Point and complete the remaining questions on the Worksheet.
16. Track the location of Plasma on the worksheet within box following **Figure 3**, specify the freezer, row & column in CDMS. Mark-up the freezer box as shown below before placing the box in your freezer.

Figure 3: Box Configuration



8. Shipping:

A. Shipping Overview:

Specimens which have been entered into the CDMS database as part of TRACER will be shipped to the Frederick National Laboratory for Cancer Research (FNLCR) in Frederick, MD (aka NCI-Frederick) for long term storage and distribution to approved analytic labs.

B. Preparing for Shipment and Shipping Schedule

Step 1. Only ship Monday – Wednesday

Step 2. Assess which boxes of specimens will be shipped and complete the following steps In CDMS >Specimens:

a. Check the No Specimen PQs Report for a list of specimens that are missing answers to the processing questions, see MOP **Figure 5.18** in **Section 5.7.5** of MOP

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 **Figure 5.19** in **Section 5.7.6** of MOP

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 CDMS.

Step 3. The shipping sites will receive a shipping box from FNLCR. To request shippers please create an Inquiry to the Coordinating Center in CDMS.

Step 4. The shipping label (airbill) is included in the shipping box.

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 must 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.

C. "Shipping" Specimen Boxes in CDMS

1. To "ship the specimens in CDMS electronically", you must have a list of the Box ID Numbers (assigned during original key entry) to be shipped and the UPS/FedEx tracking number to be used. For complete instructions please review **Section 5.6** of 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) (**Figure 4**)

Figure 4: Box Diagram

	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 CDMS" 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 CDMS.

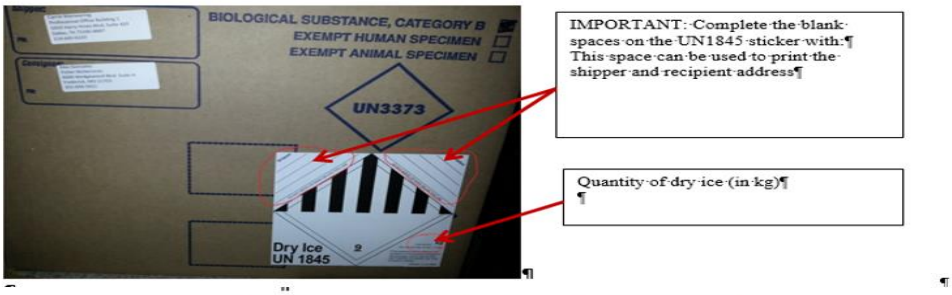
3. When specimens are successfully "shipped in CDMS" a shipping manifest is created in CDMS, and an automated e-mail notification is sent to the FNLCR to notify them of an incoming shipment, and it also includes a shipping manifest and courier tracking number. The shipping site should print 2 copies of the shipment manifest. Place one in the box being shipped and keep the other with the study documentation.

D. 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 watertight and pressure safe layers with absorbent material in between (**Figure 5**). Triple packaging consists of the following:
   a. A leak proof primary receptacle (i.e., Cryovial)
   b. A leak proof secondary packaging (i.e., biohazard bag + 95kPa sleeve; or 95kPa Biohazard bag)
   c. An outer rigid packaging of adequate strength for its capacity, mass and intended use (i.e., 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.

Figure 5: Shipping Labels

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 pickup on the same day. Or call to schedule a pickup 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.

E. Specimen Shipping Address & Contact Information

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

The shipping label placed on the container must have the following address:

BioProcessing and Trial Logistics
Attn: TRACER
BioProcessing Laboratory
4600 Wedgewood Blvd
Suite K
Frederick, MD 21703
(240) 586-1601
NCI-FrederickCSPBPTLStaff@mail.nih.gov