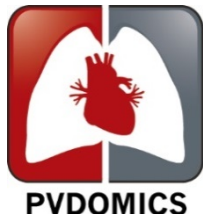


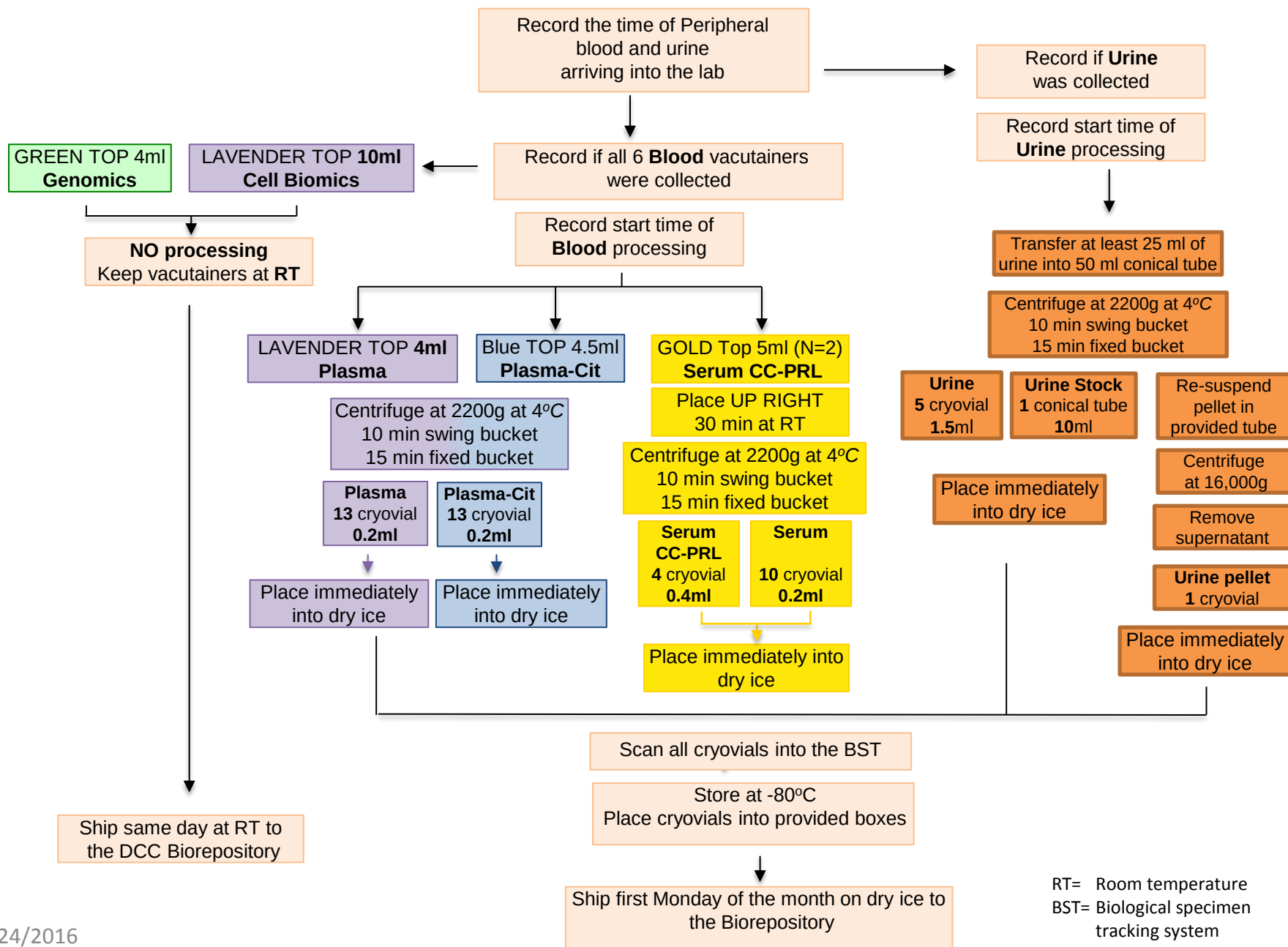
Processing, Aliquoting, Recording and Storing of Biospecimens



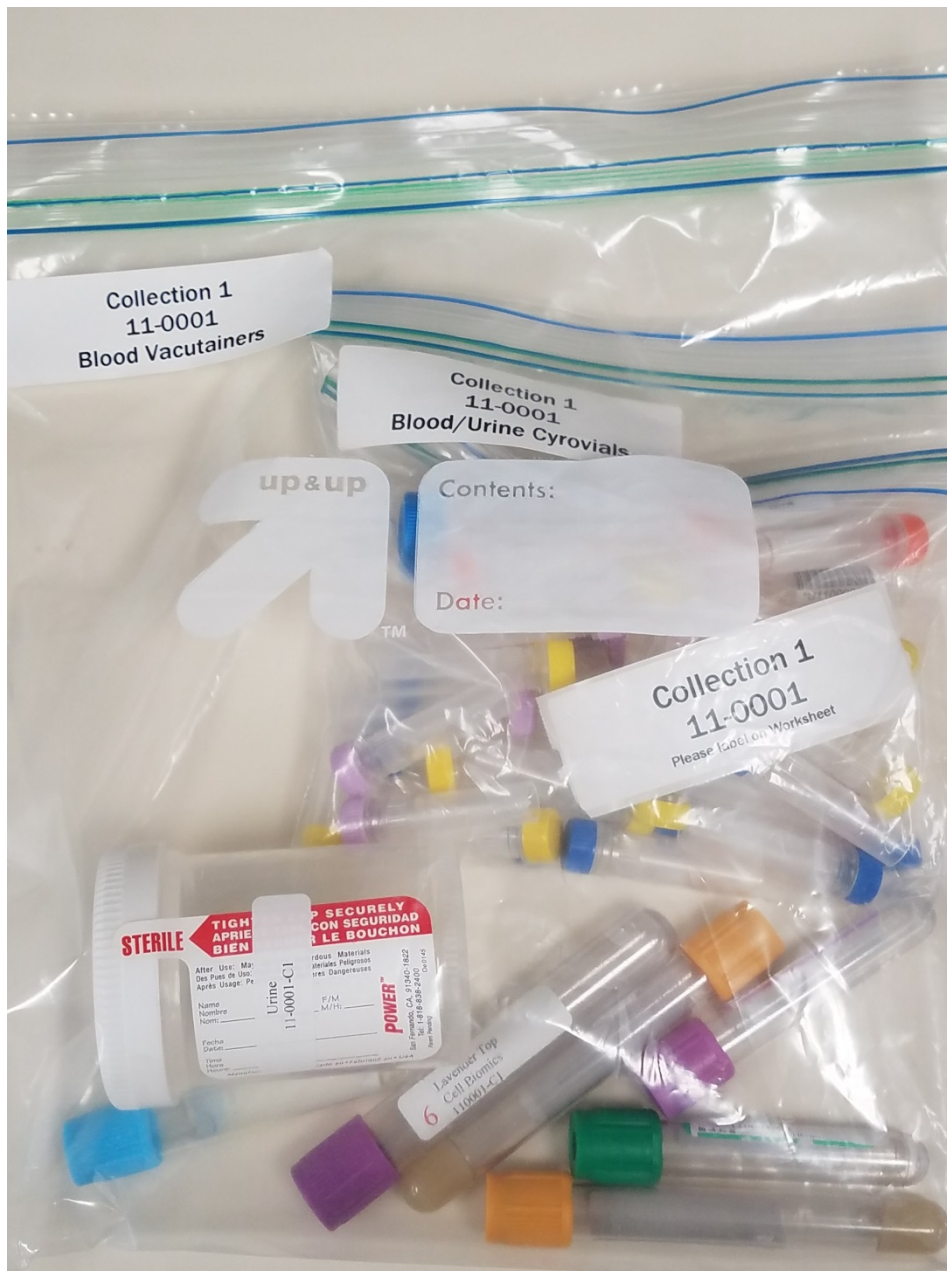
NHLBI Pulmonary Vascular Disease Phenomics Program

*Funded by the National Heart, Lung, and Blood Institute of the
National Institutes of Health with support from
the Pulmonary Hypertension Association*

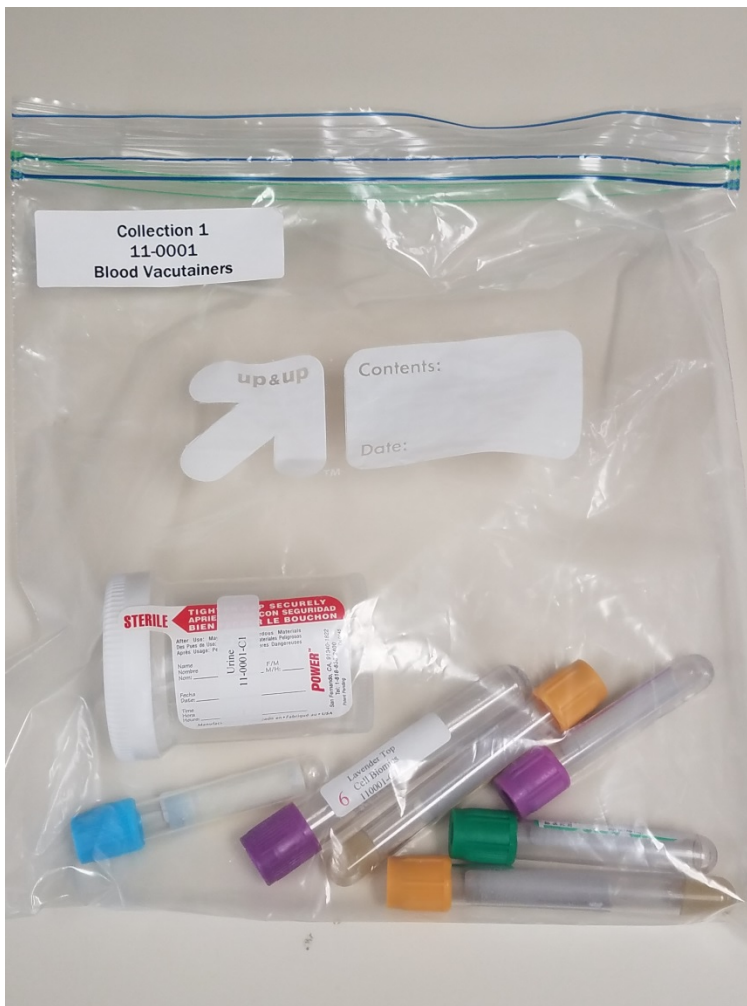
Appendix 34.1: PVDOMICS OMICS Blood and Urine Collection 1: Processing, Aliquoting, Recording and Storing of Biospecimens



RT= Room temperature
BST= Biological specimen tracking system



Collection 1 Kit:
Includes 1 bag of aliquot tubes and 1 bag of blood vacutainers with urine cup.



- Blood Vacutainers: **lavender** top 10ml cell biomics, **green** top 4ml genomics, **blue** top 4.5ml plasma-cit, **lavender** top 4ml plasma, and two **gold** top 5ml serum CC-PRL. Urine Cup



- Aliquot Tubes: 13 **lavender** tubes, 13 **blue** tubes, 4 **gold** serum CC-PRL tubes, and 10 **gold** serum tubes, 6 **orange** tubes, 15ml tube, 50 ml tube.



After blood draw, place the following tubes on wet ice:

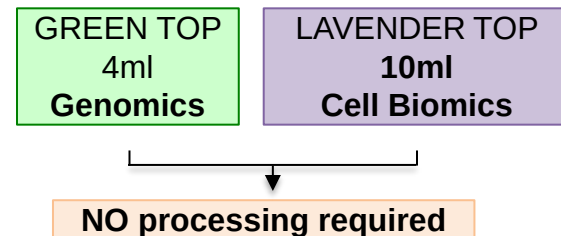
LAVENDER TOP
4ml Plasma

BLUE TOP 4.5ml
Plasma-Cit

GOLD TOP 5ml (N=2)
Serum CC-PRL



After blood draw, keep the following tubes at room temperature and set aside for separate shipment:





Place the two **Gold Top Serum CC-PRL** tubes upright at room temperature for 30 minutes.

- Next, print and begin to fill out form 301 (#1-7)

Revision of 09/01/2016



Form #301
Page 1 of 2

PVDOMICS STUDY Center Blood Collection 1 – Processing Sample Form #301

Instructions: Person processing blood should be familiar with the PVDOMICS blood processing MOP Chapter 34.

1	1	0	0	0	1
---	---	---	---	---	---

1. Identification Number

--	--

2. Alphacode

0	9	0	7	2	0	1	6
---	---	---	---	---	---	---	---

3. Processing Date (mm/dd/yyyy)

Collection 1
11-0001



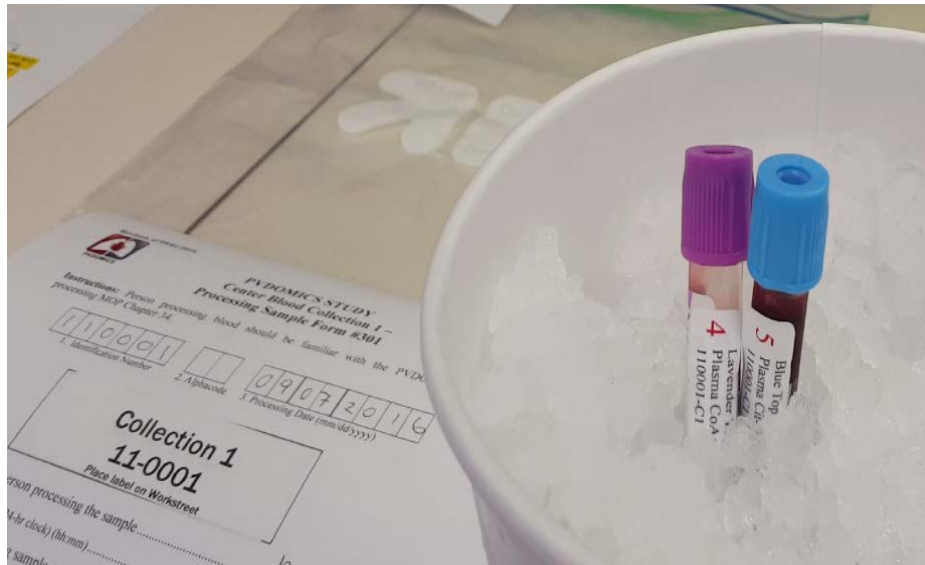
Place bag label here,
and the ID number
should match the
number on the sticker

4. Username of the person processing the sample baickes
5. Time received at lab (24-hr clock) (hh:mm) 13:58
6. Time of start of processing sample (24-hr clock) (hh:mm) 14:02
7. Date sample frozen (mm/dd/yyyy) 09/07/2016

- Then fill out #8 and #9

Type of tube(s)	8. Was the required volume of blood collected? 0=No, 1=Yes, 8=Not collected	9. Was the specimen placed on ice before processing? 0=No, 1=Yes, 8=Not collected	10. Start time of processing sample (24-hr clock) (hh:mm)	11. Specimen condition 0=Normal, 1=Hemolysis, 2=Milky	12. Time specimen frozen in dry ice (24-hr clock) (hh:mm)	13. Time specimen placed in -70°C/-80°C freezer (24-hr clock) (hh:mm)
a. Gold Top SST 5ml	<u>1</u>	<u>1</u>	<u>14:33</u>	<u>0</u>	<u>14:45</u>	<u>14:50</u>
b. Gold Top SST 5ml	<u>1</u>	<u>1</u>	<u>14:33</u>	<u>0</u>	<u>14:45</u>	<u>14:50</u>
c. Lavender Top EDTA Plasma 4ml	<u>1</u>	<u>1</u>	<u>14:05</u>	<u>0</u>	<u>14:26</u>	<u>14:50</u>
d. Blue Top Na-Citrate 4.5ml	<u>1</u>	<u>1</u>	<u>14:05</u>	<u>0</u>	<u>14:26</u>	<u>14:50</u>

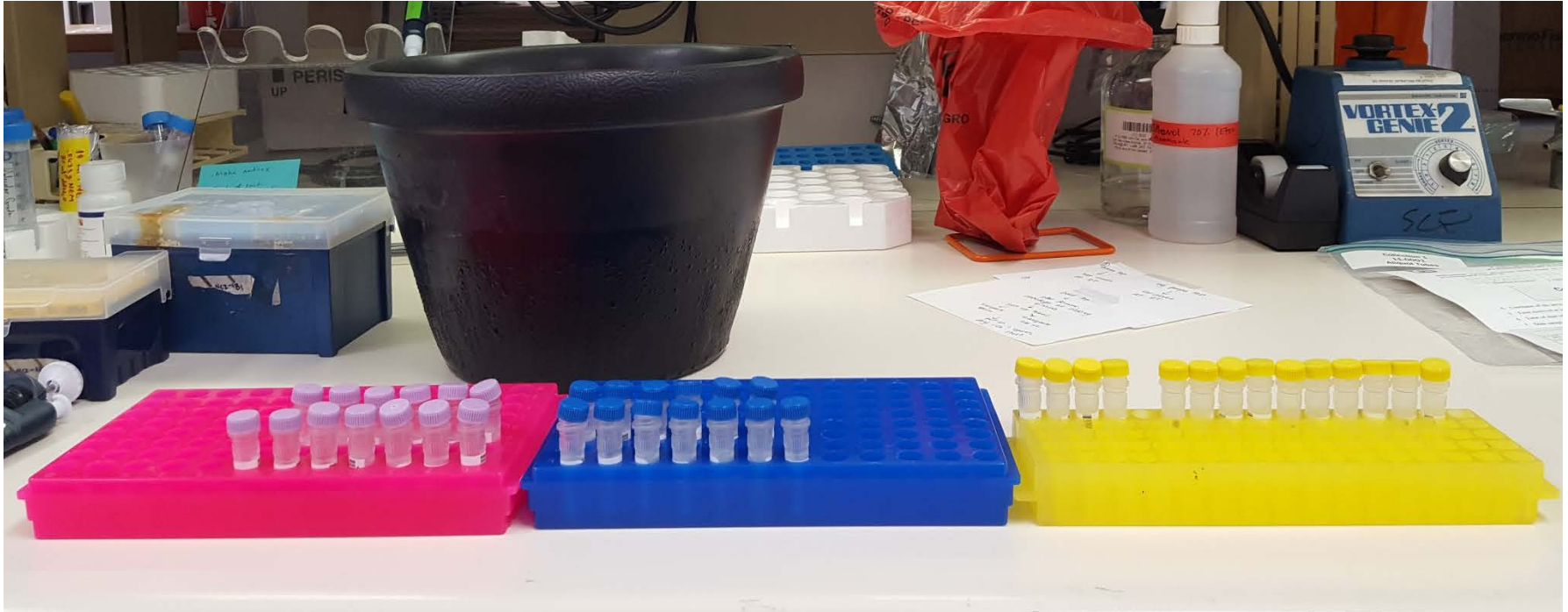
- Write the processing start time on the form for the **blue top** (4.5ml) and **lavender top** (4ml) samples.



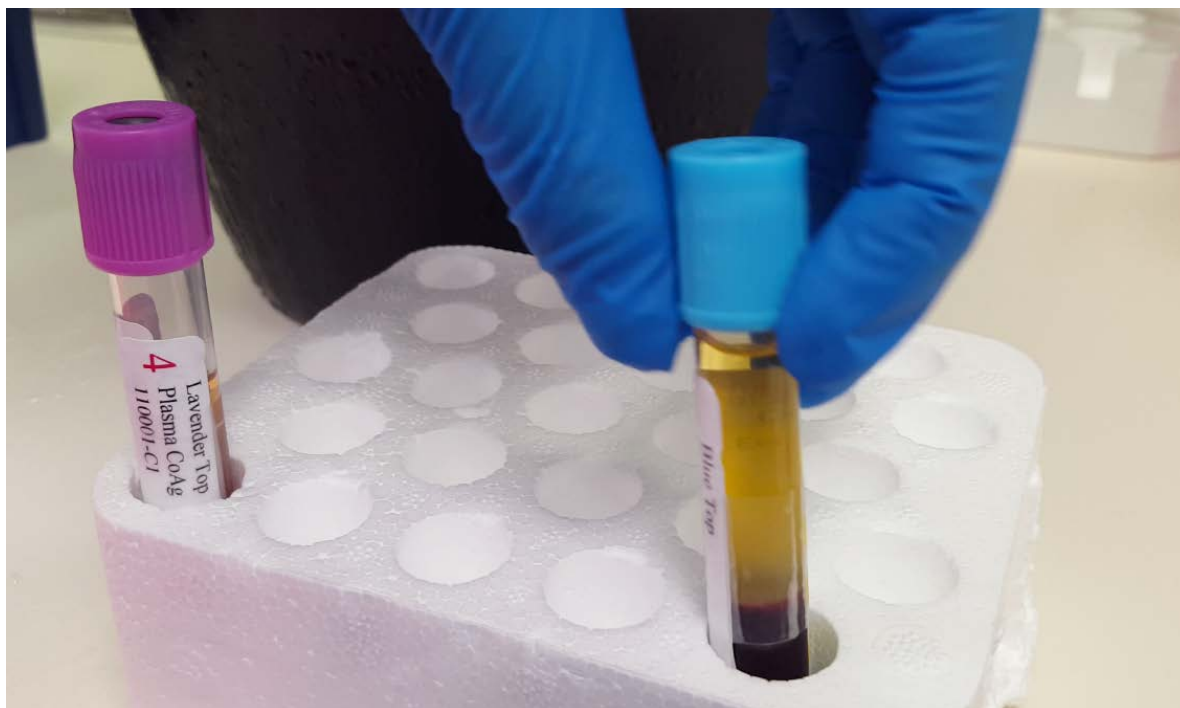
Type of tube(s)	8. Was the required volume of blood collected? 0=No, 1=Yes, 8=Not collected	9. Was the specimen placed on ice before processing? 0=No, 1=Yes, 8=Not collected	10. Start time of processing sample (24-hr clock) (hh:mm)
a. Gold Top SST 5ml	<u>1</u>	<u>1</u>	14:33
b. Gold Top SST 5ml	<u>1</u>	<u>1</u>	14:33
c. Lavender Top EDTA Plasma 4ml	<u>1</u>	<u>1</u>	14:05
d. Blue Top Na-Citrate 4.5ml	<u>1</u>	<u>1</u>	14:05



- Centrifuge the **blue top** and **lavender top** tubes at 2200g at 4° for 10 minutes (swing bucket) or 15 minutes (fixed bucket).



- While waiting for the centrifuge step, set up aliquot tubes and get dry ice ready.

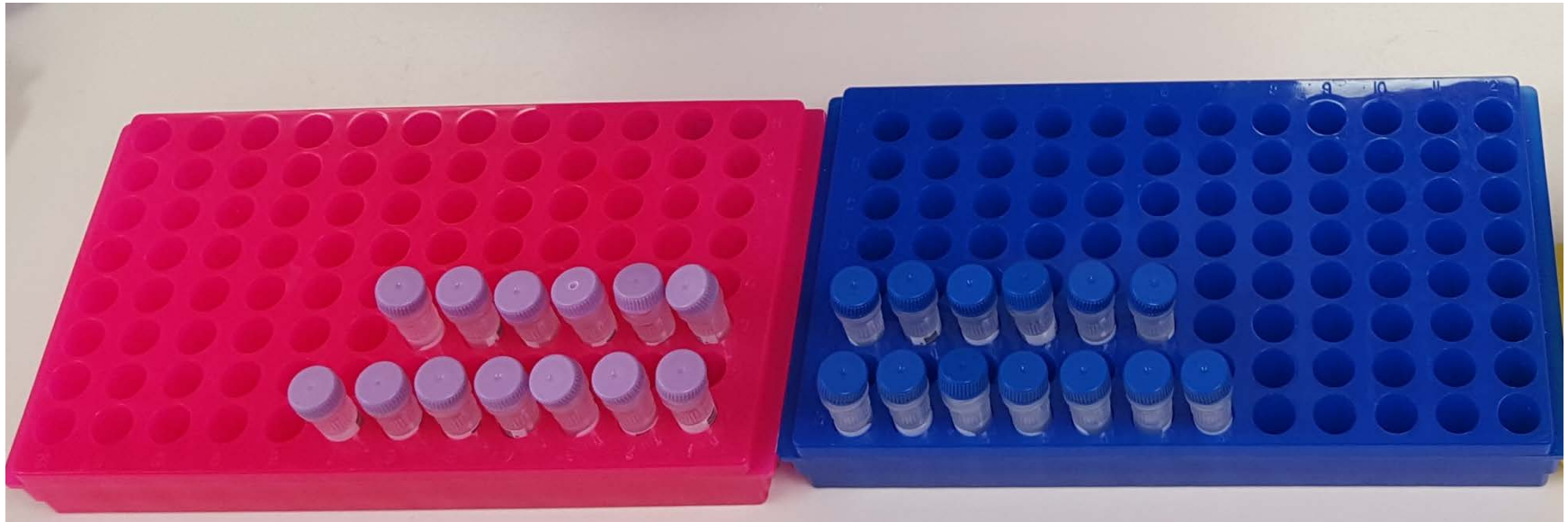


- After the centrifugation step, note the quality of the specimen on the form for both the **blue** and **lavender** top tubes.

Type of tube(s)	8. Was the required volume of blood collected? 0=No, 1=Yes, 8=Not collected	9. Was the specimen placed on ice before processing? 0=No, 1=Yes, 8=Not collected	10. Start time of processing sample (24-hr clock) (hh:mm)	11. Specimen condition 0=Normal, 1=Hemolysis, 2=Milky	12. Time specimen frozen in dry ice (24-hr clock) (hh:mm)	13. Time specimen placed in -70°C/-80°C freezer (24-hr clock) (hh:mm)
a. Gold Top SST 5ml	<u>1</u>	<u>1</u>	<u>14:33</u>	<u>0</u>	<u>14:45</u>	<u>14:50</u>
b. Gold Top SST 5ml	<u>1</u>	<u>1</u>	<u>14:33</u>	<u>0</u>	<u>14:45</u>	<u>14:50</u>
c. Lavender Top EDTA Plasma 4ml	<u>1</u>	<u>1</u>	<u>14:05</u>	<u>0</u>	<u>14:26</u>	<u>14:50</u>
d. Blue Top Na-Citrate 4.5ml	<u>1</u>	<u>1</u>	<u>14:05</u>	<u>0</u>	<u>14:26</u>	<u>14:50</u>

Lavender Top Tube

Blue Top Tube



Plasma
13 aliquot
0.2ml into each tube



Place immediately
into dry ice

Plasma-Cit
13 aliquot
0.2ml into each tube



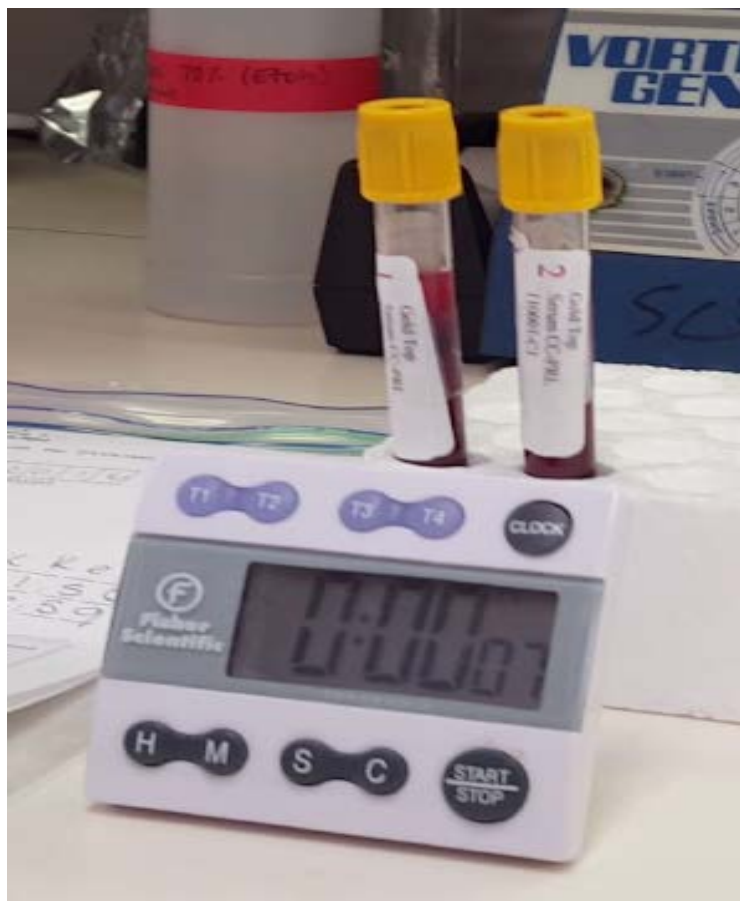
Place immediately
into dry ice



- **Note: If the last tube has less than 0.2ml, put a dot on the top of the tube.**
- **This will be done for the last lavender, blue, and gold 0.2ml aliquots.**

Type of tube(s)	8. Was the required volume of blood collected? 0=No, 1=Yes, 8=Not collected	9. Was the specimen placed on ice before processing? 0=No, 1=Yes, 8=Not collected	10. Start time of processing sample (24-hr clock) (hh:mm)	11. Specimen condition 0=Normal, 1=Hemolysis, 2=Milky	12. Time specimen frozen in dry ice (24-hr clock) (hh:mm)	13. Time specimen placed in -70°C/-80°C freezer (24-hr clock) (hh:mm)
a. Gold Top SST 5ml	<u>1</u>	<u>1</u>	<u>14:33</u>	<u>0</u>	<u>14:45</u>	<u>14:50</u>
b. Gold Top SST 5ml	<u>1</u>	<u>1</u>	<u>14:33</u>	<u>0</u>	<u>14:45</u>	<u>14:50</u>
c. Lavender Top EDTA Plasma 4ml	<u>1</u>	<u>1</u>	<u>14:05</u>	<u>0</u>	<u>14:26</u>	<u>14:50</u>
d. Blue Top Na-Citrate 4.5ml	<u>1</u>	<u>1</u>	<u>14:05</u>	<u>0</u>	<u>14:26</u>	<u>14:50</u>

- **On the form, make note of the time when the blue top and lavender top specimens were placed into dry ice.**



Type of tube(s)	8. Was the required volume of blood collected? 0=No, 1=Yes, 8=Not collected	9. Was the specimen placed on ice before processing? 0=No, 1=Yes, 8=Not collected	10. Start time of processing sample (24-hr clock) (hh:mm)
a. Gold Top SST 5ml	<u>1</u>	<u>1</u>	<u>14:33</u>
b. Gold Top SST 5ml	<u>1</u>	<u>1</u>	<u>14:33</u>
c. Lavender Top EDTA Plasma 4ml	<u>1</u>	<u>1</u>	<u>14:05</u>
d. Blue Top Na-Citrate 4.5ml	<u>1</u>	<u>1</u>	<u>14:05</u>

After the **gold top** tubes have been at room temperature for 30 minutes, write the processing start time on the form and centrifuge the samples.



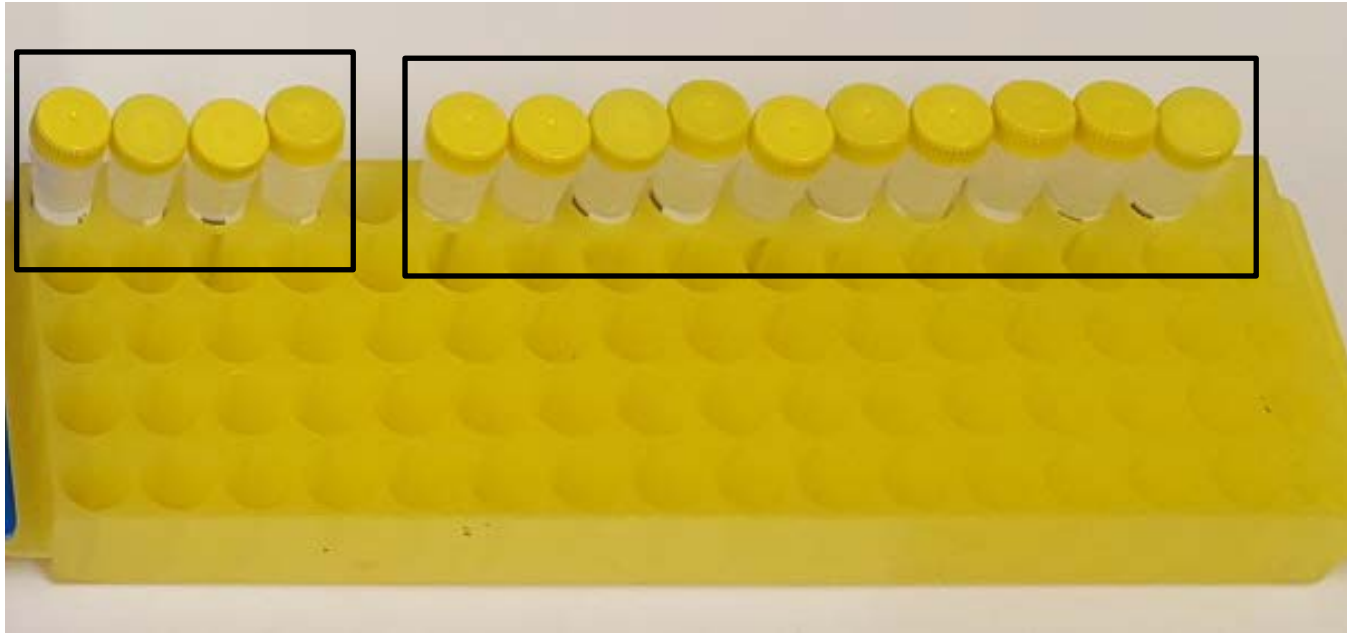
- Centrifuge the same as before at 2200g at 4° for 10 minutes (swing bucket) or 15 minutes (fixed bucket).



- As before, after centrifugation, note the quality of the specimen on the form for the **gold top** tubes.

Type of tube(s)	8. Was the required volume of blood collected? 0=No, 1=Yes, 8=Not collected	9. Was the specimen placed on ice before processing? 0=No, 1=Yes, 8=Not collected	10. Start time of processing sample (24-hr clock) (hh:mm)	11. Specimen condition 0=Normal, 1=Hemolysis, 2=Milky
a. Gold Top SST 5ml	<u>1</u>	<u>1</u>	<u>14:33</u>	<u>0</u>
b. Gold Top SST 5ml	<u>1</u>	<u>1</u>	<u>14:33</u>	<u>0</u>
c. Lavender Top EDTA Plasma 4ml	<u>1</u>	<u>1</u>	<u>14:05</u>	<u>0</u>
d. Blue Top Na-Citrate 4.5ml	<u>1</u>	<u>1</u>	<u>14:05</u>	<u>0</u>

Gold Top Tubes



Serum CC-PRL
4 aliquot
0.4ml into each tube

Serum
10 aliquot
0.2ml into each tube

Place immediately
into dry ice

- On the form, make note of the time when the **gold top** tube specimens were placed into dry ice.

Type of tube(s)	8. Was the required volume of blood collected? 0=No, 1=Yes, 8=Not collected	9. Was the specimen placed on ice before processing? 0=No, 1=Yes, 8=Not collected	10. Start time of processing sample (24-hr clock) (hh:mm)	11. Specimen condition 0=Normal, 1=Hemolysis, 2=Milky	12. Time specimen frozen in dry ice (24-hr clock) (hh:mm)	13. Time specimen placed in -70°C/-80°C freezer (24-hr clock) (hh:mm)
a. Gold Top SST 5ml	<u>1</u>	<u>1</u>	<u>14:33</u>	<u>0</u>	<u>14:45</u>	<u>14:50</u>
b. Gold Top SST 5ml	<u>1</u>	<u>1</u>	<u>14:33</u>	<u>0</u>	<u>14:45</u>	<u>14:50</u>
c. Lavender Top EDTA Plasma 4ml	<u>1</u>	<u>1</u>	<u>14:05</u>	<u>0</u>	<u>14:26</u>	<u>14:50</u>
d. Blue Top Na-Citrate 4.5ml	<u>1</u>	<u>1</u>	<u>14:05</u>	<u>0</u>	<u>14:26</u>	<u>14:50</u>

- Then fill in #14-17 regarding the number of cryovials.

14. Number of cryovials of 400ul Serum-CC-PRL	<u>004</u>
15. Number of cryovials of 200ul Serum	<u>010</u>
16. Number of cryovials of 200ul Plasma	<u>013</u>
17. Number of cryovials of 200ul Plasma-Cit	<u>013</u>

Note: Place a black dot with permanent marker on the screw cap if the volume is less than 200 ul

Type of tube(s)	8. Was the required volume of blood collected? 0=No, 1=Yes, 8=Not collected	9. Was the specimen placed on ice before processing? 0=No, 1=Yes, 8=Not collected	10. Start time of processing sample (24-hr clock) (hh:mm)	11. Specimen condition 0=Normal, 1=Hemolysis, 2=Milky	12. Time specimen frozen in dry ice (24-hr clock) (hh:mm)	13. Time specimen placed in -70°C/-80°C freezer (24-hr clock) (hh:mm)
a. Gold Top SST 5ml	<u>1</u>	<u>1</u>	<u>14:33</u>	<u>0</u>	<u>14:45</u>	<u>14:50</u>
b. Gold Top SST 5ml	<u>1</u>	<u>1</u>	<u>14:33</u>	<u>0</u>	<u>14:45</u>	<u>14:50</u>
c. Lavender Top EDTA Plasma 4ml	<u>1</u>	<u>1</u>	<u>14:05</u>	<u>0</u>	<u>14:26</u>	<u>14:50</u>
d. Blue Top Na-Citrate 4.5ml	<u>1</u>	<u>1</u>	<u>14:05</u>	<u>0</u>	<u>14:26</u>	<u>14:50</u>

- On the form, make note of the time when the all of the specimens were places in the freezer.

Revision of 09/01/2016 ID _____ AC ____ Date of processing ____/____/____

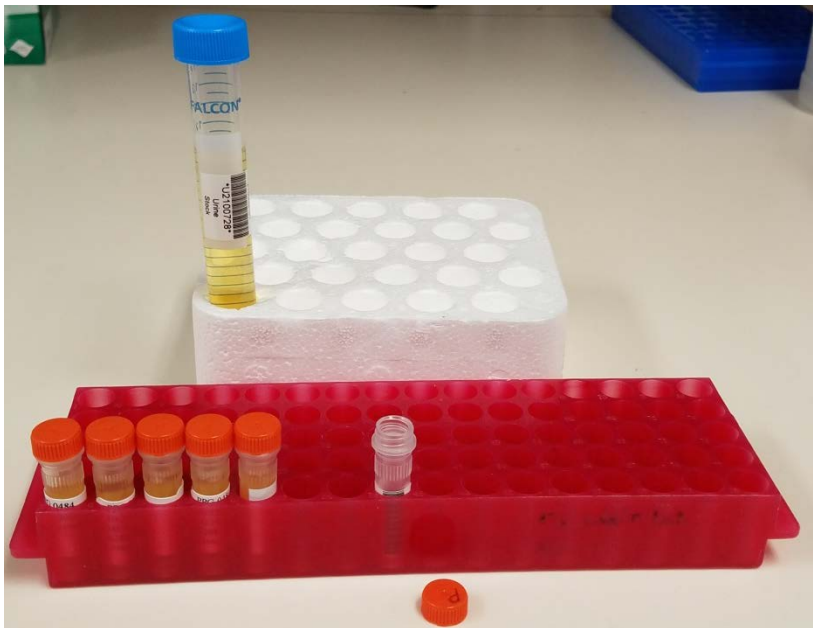
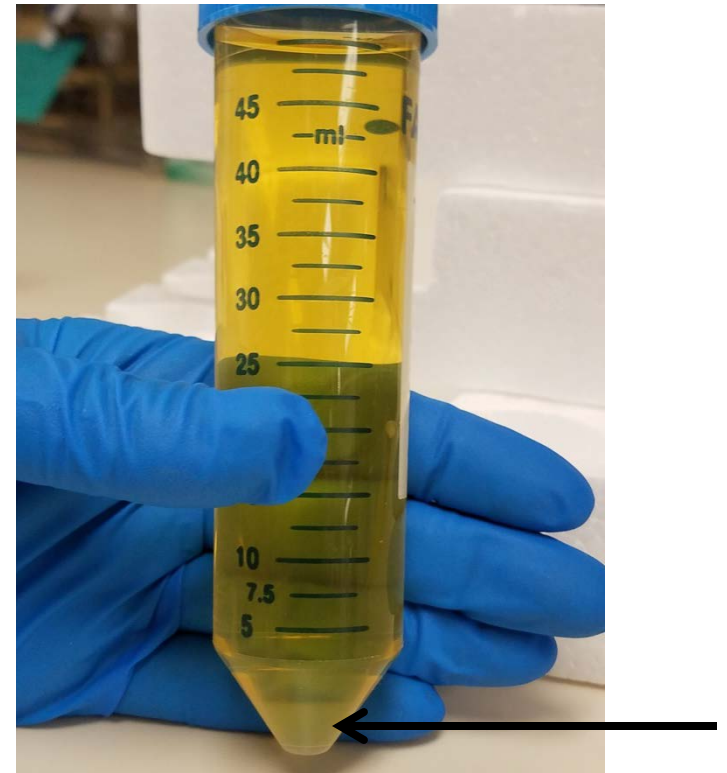
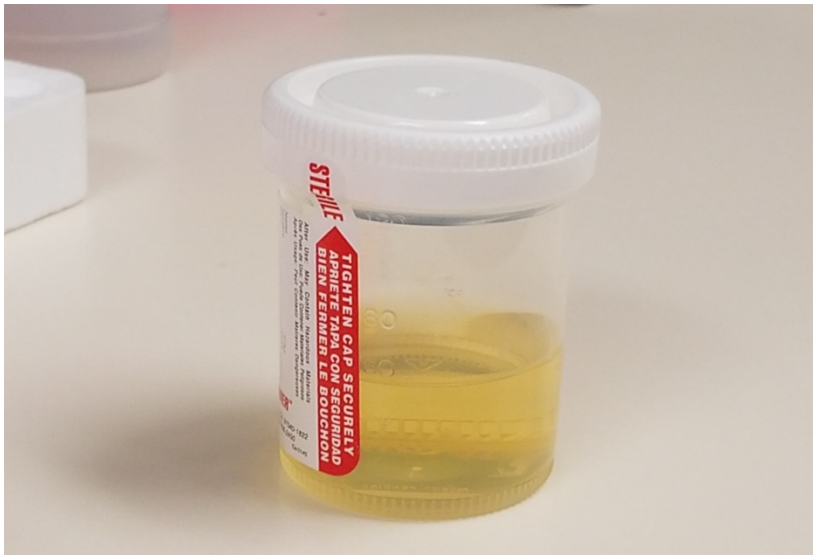
Form #301
Page 2 of 2

18. Were any issues experienced during processing (0=No, 1=Yes) 0

Comments (will not be recorded in the database):

- Lastly, fill out the remainder of the form, make note of any issues, and write in the date the form was completed.

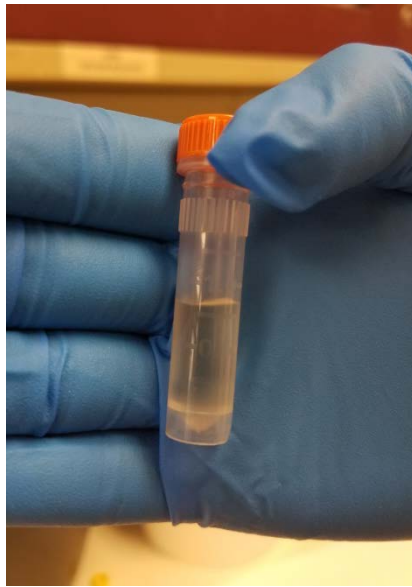
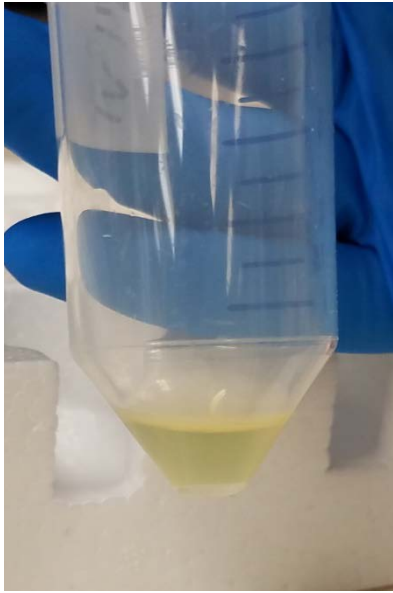
200. Date form completed (mm/dd/yyyy) 09, 07, 2016



Transfer Urine to 50ml to spin

You should see a small pellet at the bottom

Aliquot 1.5ml of supernatant
into 5 cyrovials and 10 mL to conical tube



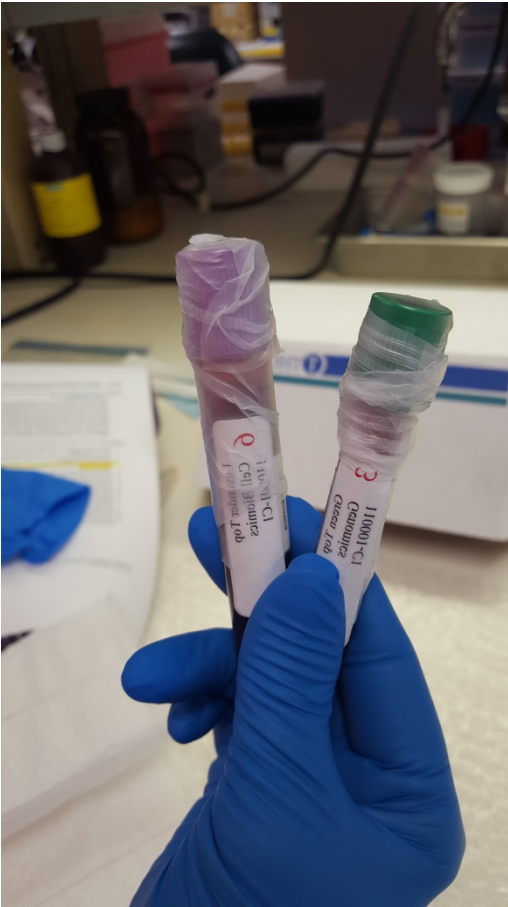
Pipet off remaining supernatant

Pipet pellet from bottom of 50ml into Urine Pellet tube

Spin in centrifuge

Remove supernatant and freeze in dry ice

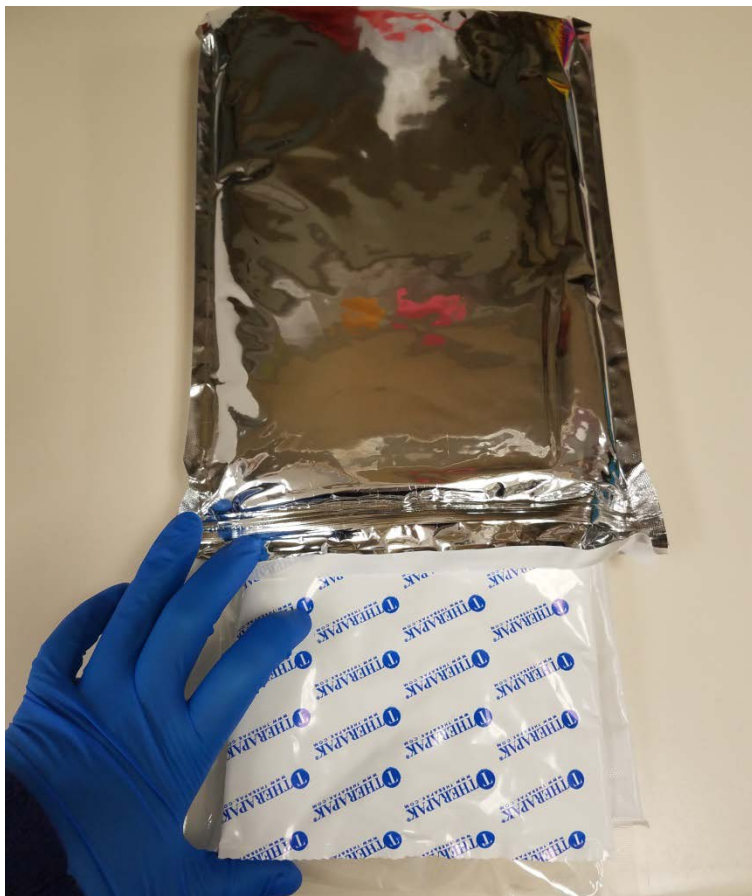
Shipping of room temperature Na-Heparin and EDTA tubes



Wrap tubes for same day shipment in parafilm

Place tubes in absorbent pouch





Slide gel pack into insulated envelope

Include contents list

Close package and place UN3373 label and shipping document on box



Collection 2: Processing, Aliquoting, Recording and Storing of Biospecimens

PVDOMICS

Appendix 34.2: PVDOMICS OMICS Blood Collection 2: Processing, Aliquoting, Recording and Storing of Biospecimens

Venous Blood
Systemic Artery "Syst Art" Blood *
Pulmonary Capillary "Wedge" Blood
Pulmonary Artery "Mix" Blood

REST

Record the time of blood
arriving into the lab

Record start time of blood
processing

Record if all 10 vacutainers
were collected

Process the 4 RED Tops (2.5ml)
Rest-Venous Genomics
Rest-Mix Genomics
Rest-Wedge Genomics
Rest-Syst Art Genomics*

Place UP RIGHT
2 HOURS at RT

Scan Tube into BST

Place UP RIGHT
24 HOURS
at -20°C

Place UP RIGHT
at -80°C

Process LAVENDER TOPS (N=5)
Plasma Rest Venous-Omics (N=2) 10ml
Plasma Rest Mix-Omics (N=1) 4ml
Plasma Rest-Wedge-Omics (N=1) 4ml
Plasma Rest-Syst Art-Omics* (N=1) 4ml

Centrifuge at 2200g at 4°C
10 min swing bucket
15 min fixed bucket

**Plasma
Rest
Venous**

**Plasma
Rest-Venous
40 cryovial 0.2ml**

**Buffy coat
Venous
1 cryovial**

**Plasma
Rest
Wedge-Omics**

**Plasma
Rest-Wedge
11 cryovial 0.2ml**

**Plasma
Rest
Mix-Omics**

**Plasma
Rest-Mix
11 cryovial 0.2ml**

**Plasma*
Rest
Syst-Art-Omics**

**Plasma
Rest-Syst Art
11 cryovial 0.2ml**

Place immediately into dry ice

Process GOLD Top 5ml
Serum

Place UP RIGHT
30 min at RT

Centrifuge at 2200g at 4°C
10 min swing bucket
15 min fixed bucket

**Serum
Rest-Venous
10 cryovial 0.2ml**

Place immediately
into dry ice

Order of processing:

1. Place the Gold top and Red tops up right
2. Centrifuge the 5 Purple tops
3. Cryovial Purple tops
4. Centrifuge Gold top
5. Cryovial Gold top

Scan all cryovials into the BST

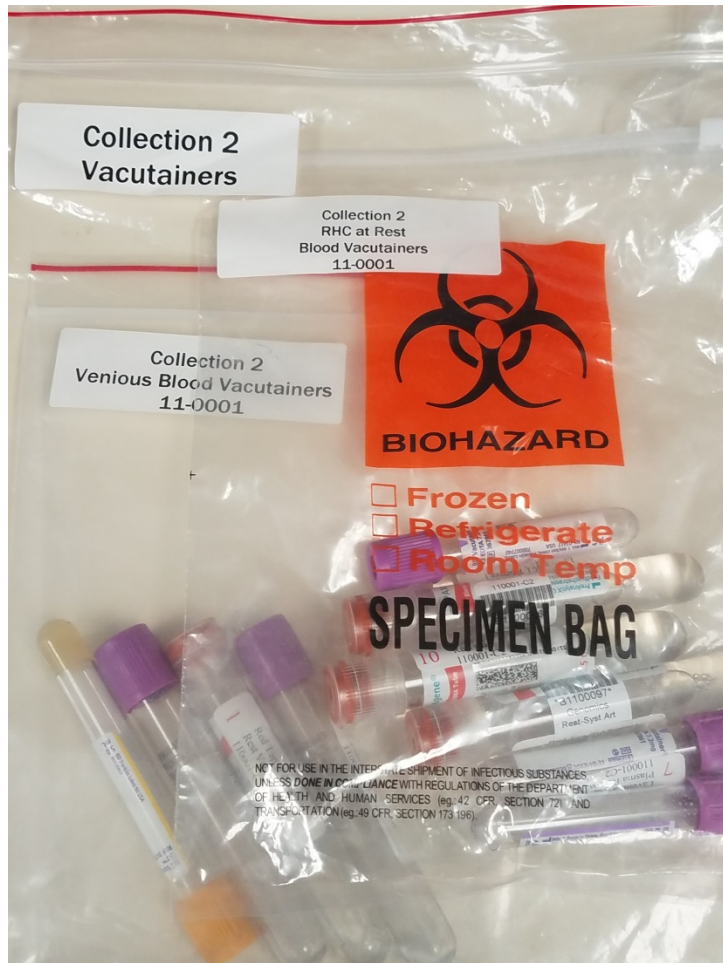
Store at -80°C
Place cryovials into provided boxes

Ship first Monday of the month on dry ice to
the Biorepository

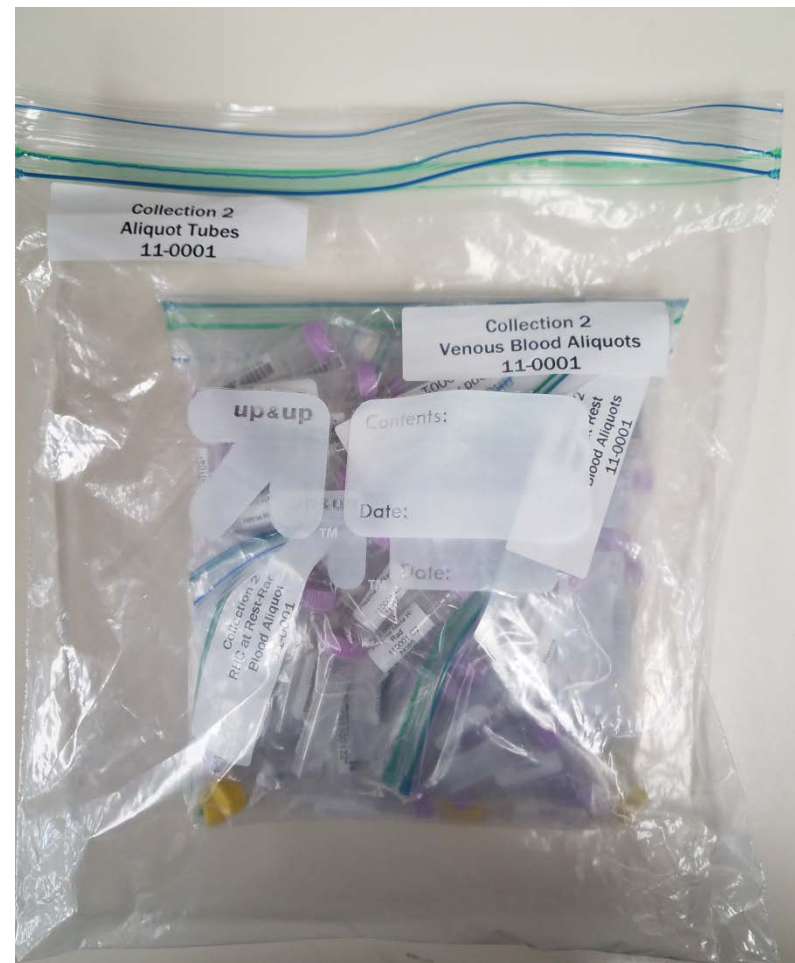
* Optional

RT= Room temperature

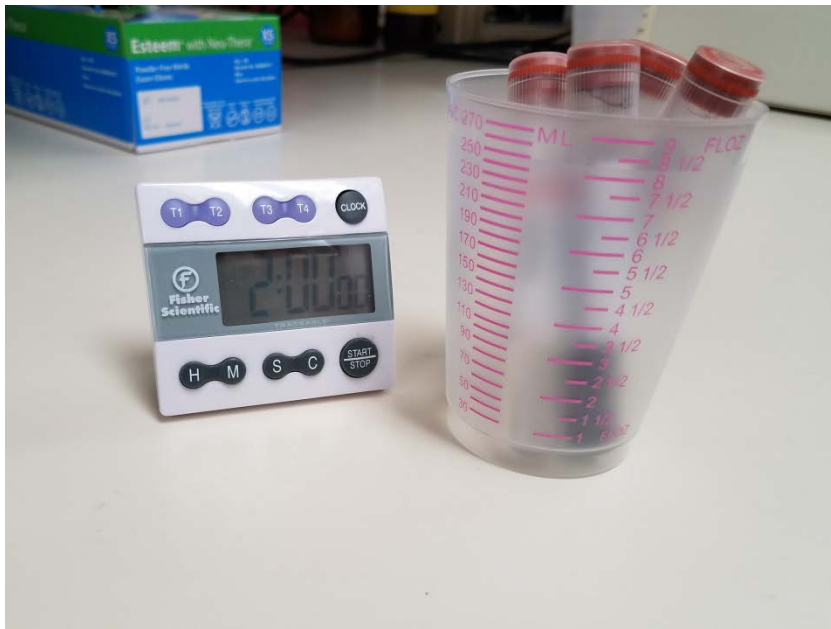
BST= Biological specimen tracking system



- Blood Vacutainers: two lavender top 10ml, three lavender top 4ml plasma, and one gold top 5ml serum, four red top tubes



- Aliquot Tubes: 73 lavender tubes, 10 gold serum tubes, and 1 clear buffy coat tube



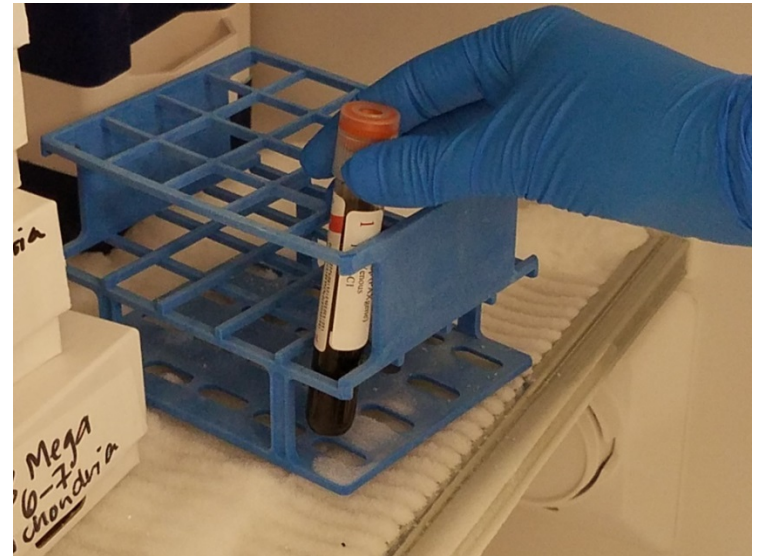
Keep the PAXgene tubes at room temperature

**RED TOP (N=4)
2.5ml PAXgene**

**Upright at Room Temp
2hrs**

**-20 Freezer
24hrs**

**-80 Freezer
Longterm**



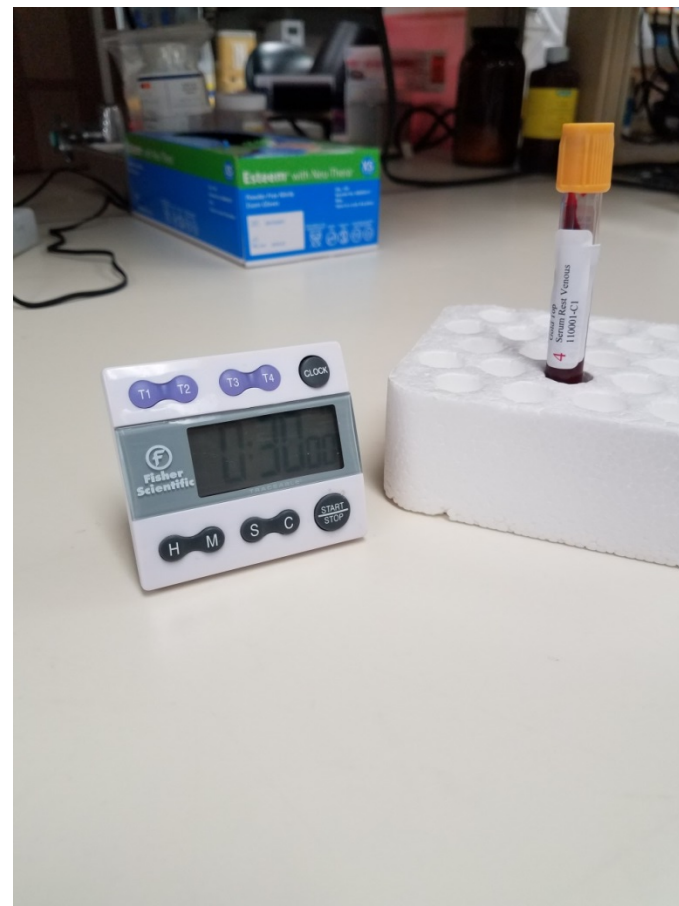
Transfer to wire rack -20 freezer
after 2 hours



After 24 hours move to -80 for long
term storage



The Serum and Plasma tubes should arrive in the lab on ice

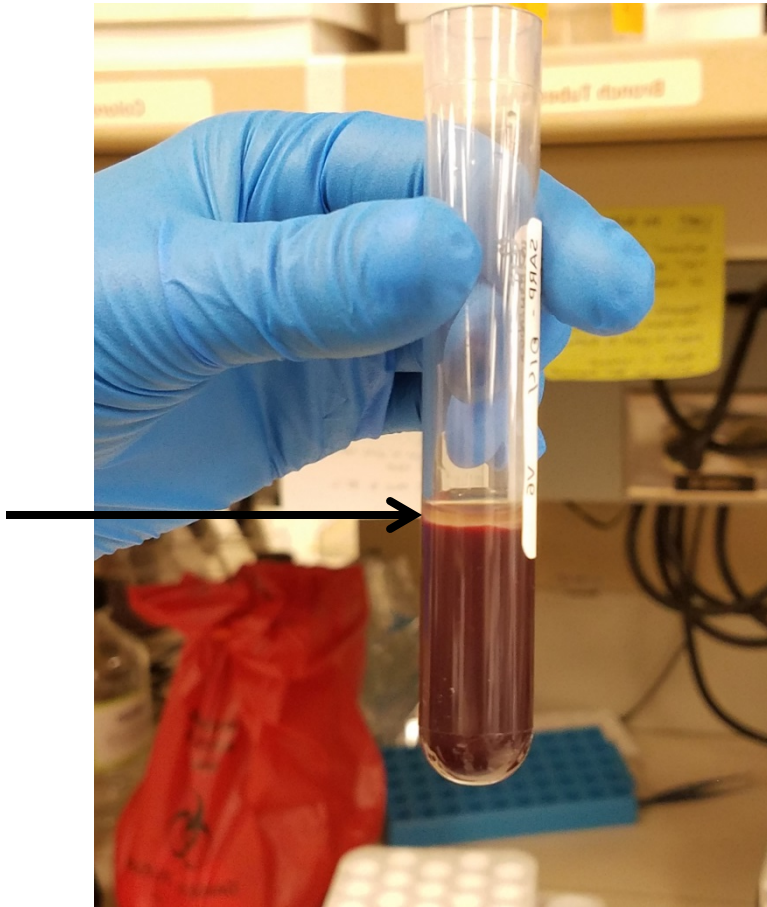


Place the **Gold Top Serum** tube upright at room temperature for 30 minutes.

LAVENDER TOP (N=2)
10ml Plasma

LAVENDER TOP (N=3)
4ml Plasma

**GOLD TOP 5ml
Serum CC-PRL**



White Buffy Coat layer is sandwiched between Plasma and RBC

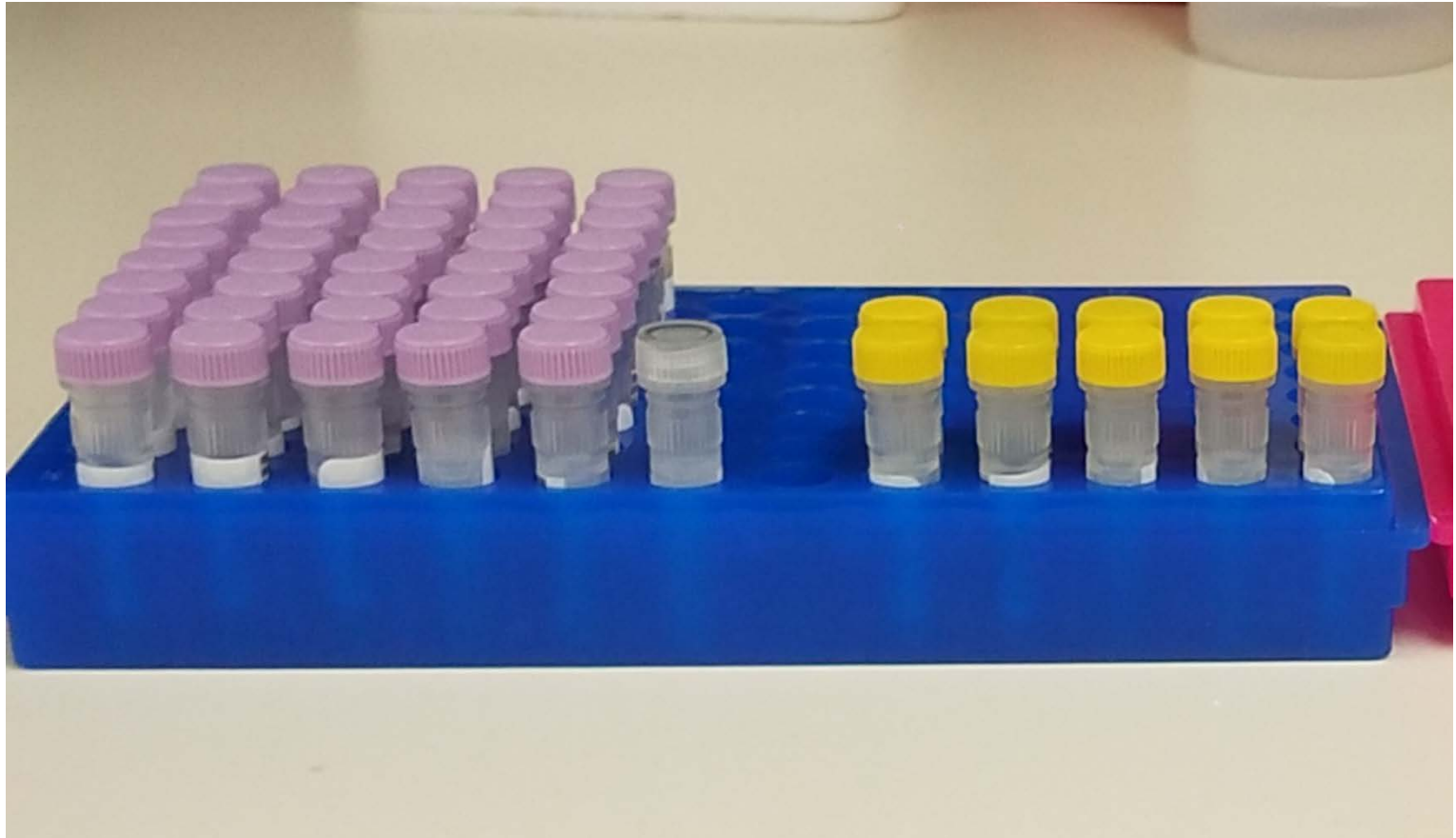
Slow decel on centrifuge makes it easier to pipet Buffy Coat



Pipet Buffy Coat using wide tip pipet, try to minimize RBC

Buffy Coat
1 aliquot

Venous Blood



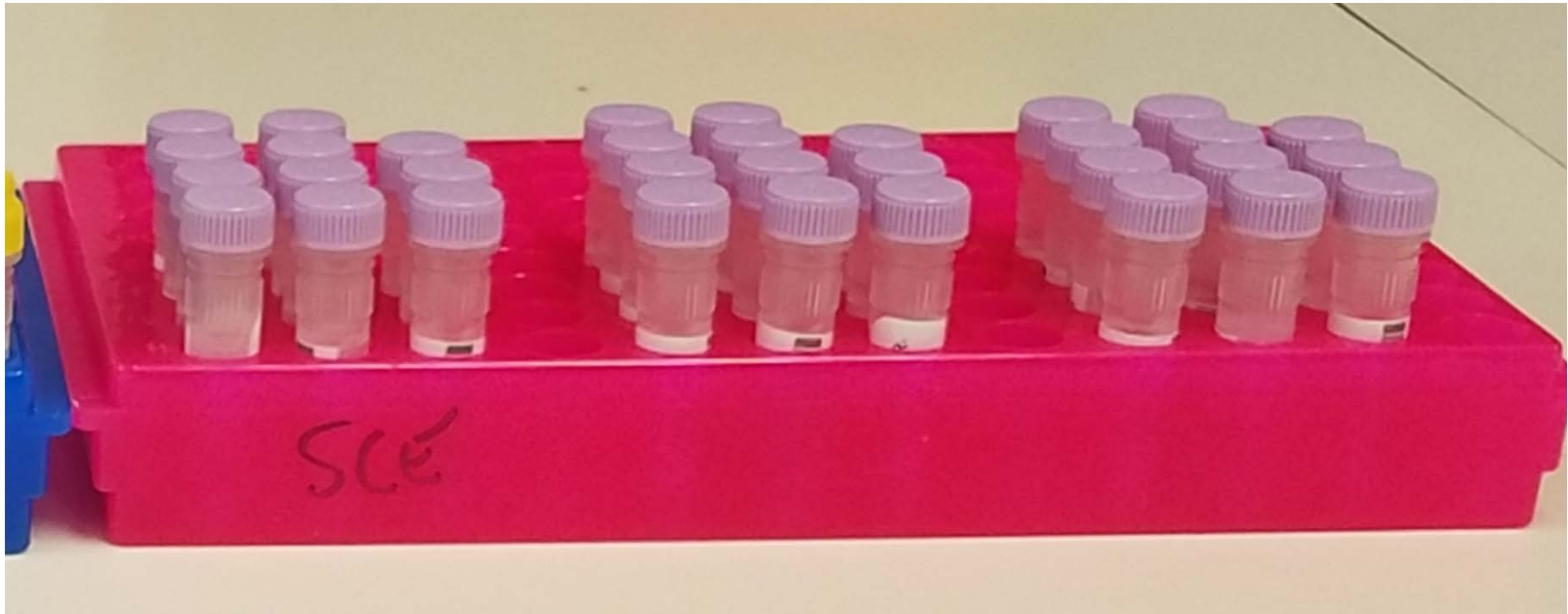
Plasma
40 aliquot
0.2ml into each tube

Buffy Coat
1 aliquot

Serum
10 aliquot
0.2ml into each tube

Place immediately
into dry ice

Mix, Wedge, and Syst Art Blood cyrovials



Plasma Mix
11 aliquot
0.2ml into each tube

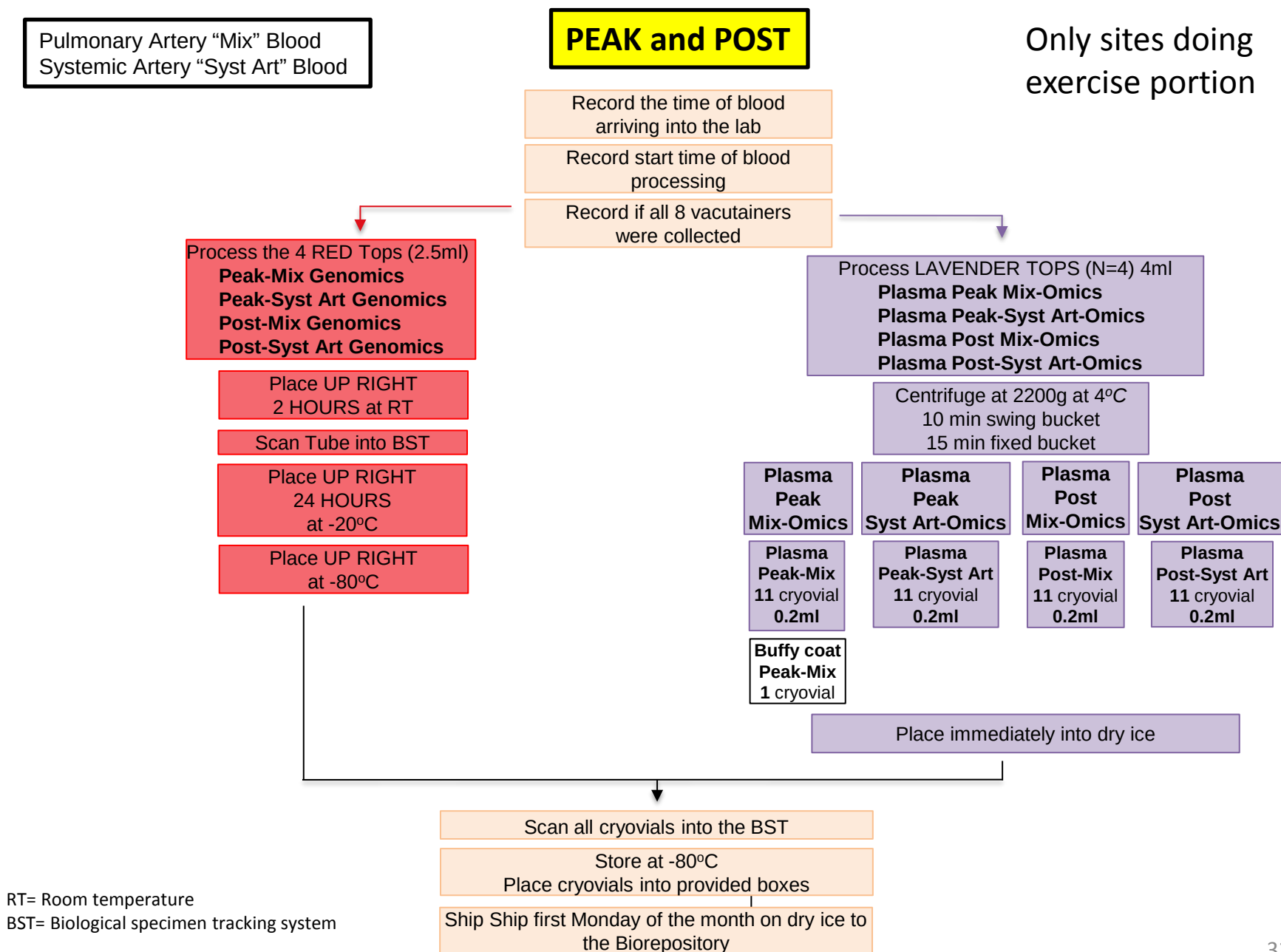
Plasma Wedge
11 aliquot
0.2ml into each tube

Plasma Syst Art*
11 aliquot
0.2ml into each tube

↓
Place immediately
into dry ice

*Optional

Appendix 34.2: PVDOMICS OMICS Blood Collection 2: Processing, Aliquoting, Recording and Storing of Biospecimens





- Blood Vacutainers: two **lavender** top 10ml, seven **lavender** top 4ml plasma, and one **gold** top 5ml serum, eight **red** top tubes



- Aliquot Tubes: 117 **lavender** tubes, 10 **gold** serum tubes, and 1 clear buffy coat tube



Keep the PAXgene tubes at room temperature

**RED TOP (N=8)
2.5ml PAXgene**

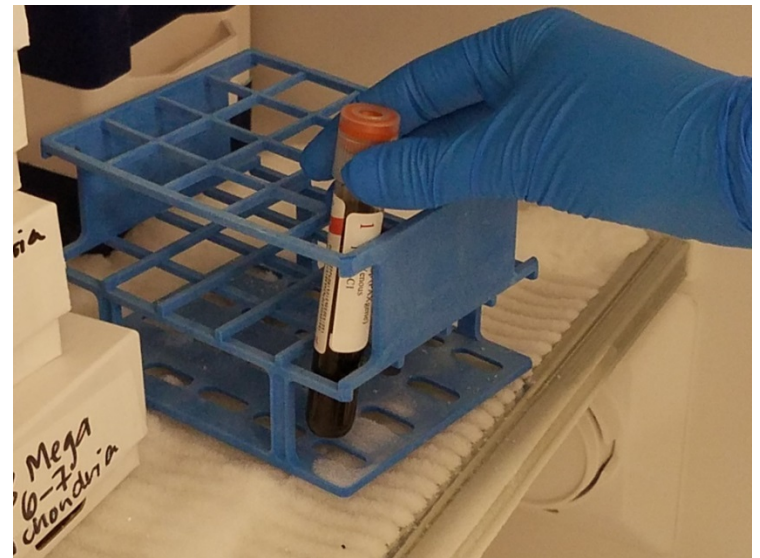
**Upright at Room Temp
2hrs**



**-20 Freezer
24hrs**



**-80 Freezer
Longterm**



Transfer to wire rack -20 freezer
after 2 hours



After 24 hours move to -80 for long
term storage

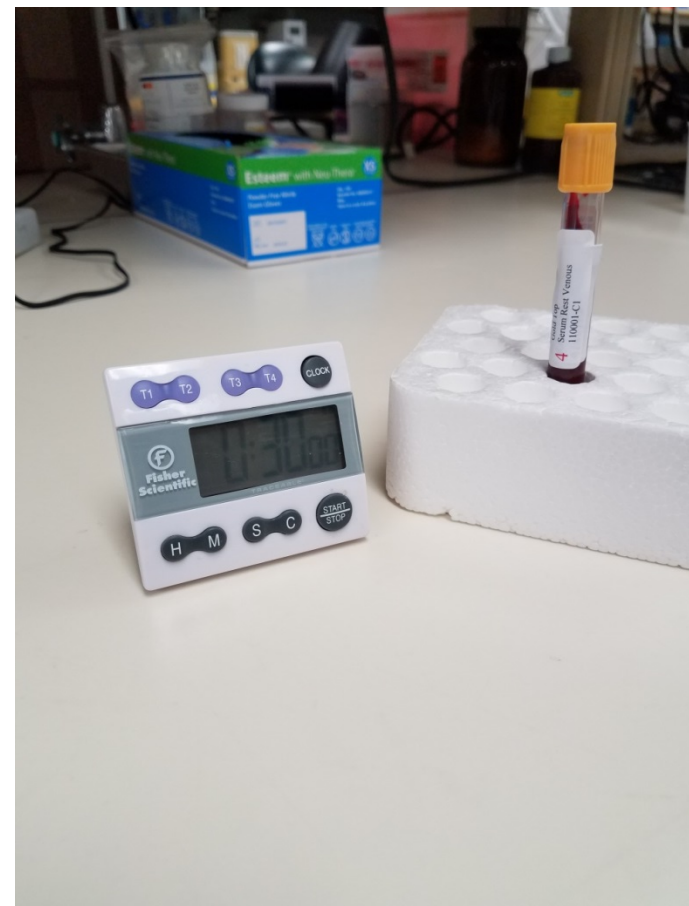


The Serum and Plasma tubes
should arrive in the lab on ice

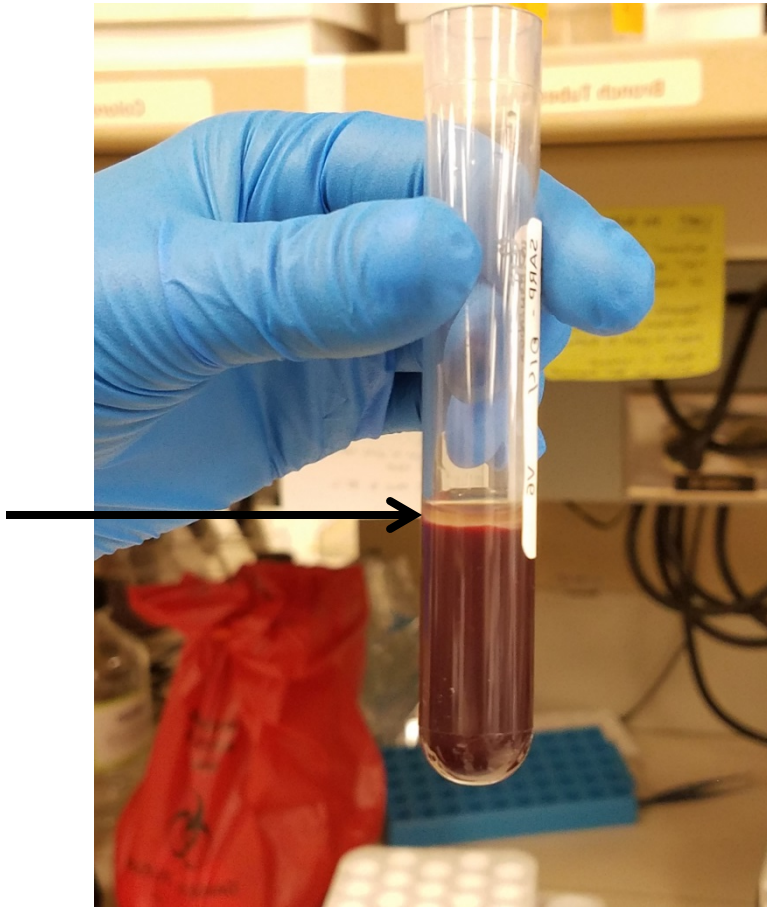
LAVENDER TOP (N=2)
10ml Plasma

LAVENDER TOP (N=7)
4ml Plasma

GOLD TOP 5ml
Serum CC-PRL



Place the **Gold Top Serum**
tube upright at room
temperature for 30 minutes.



White Buffy Coat layer is sandwiched between Plasma and RBC

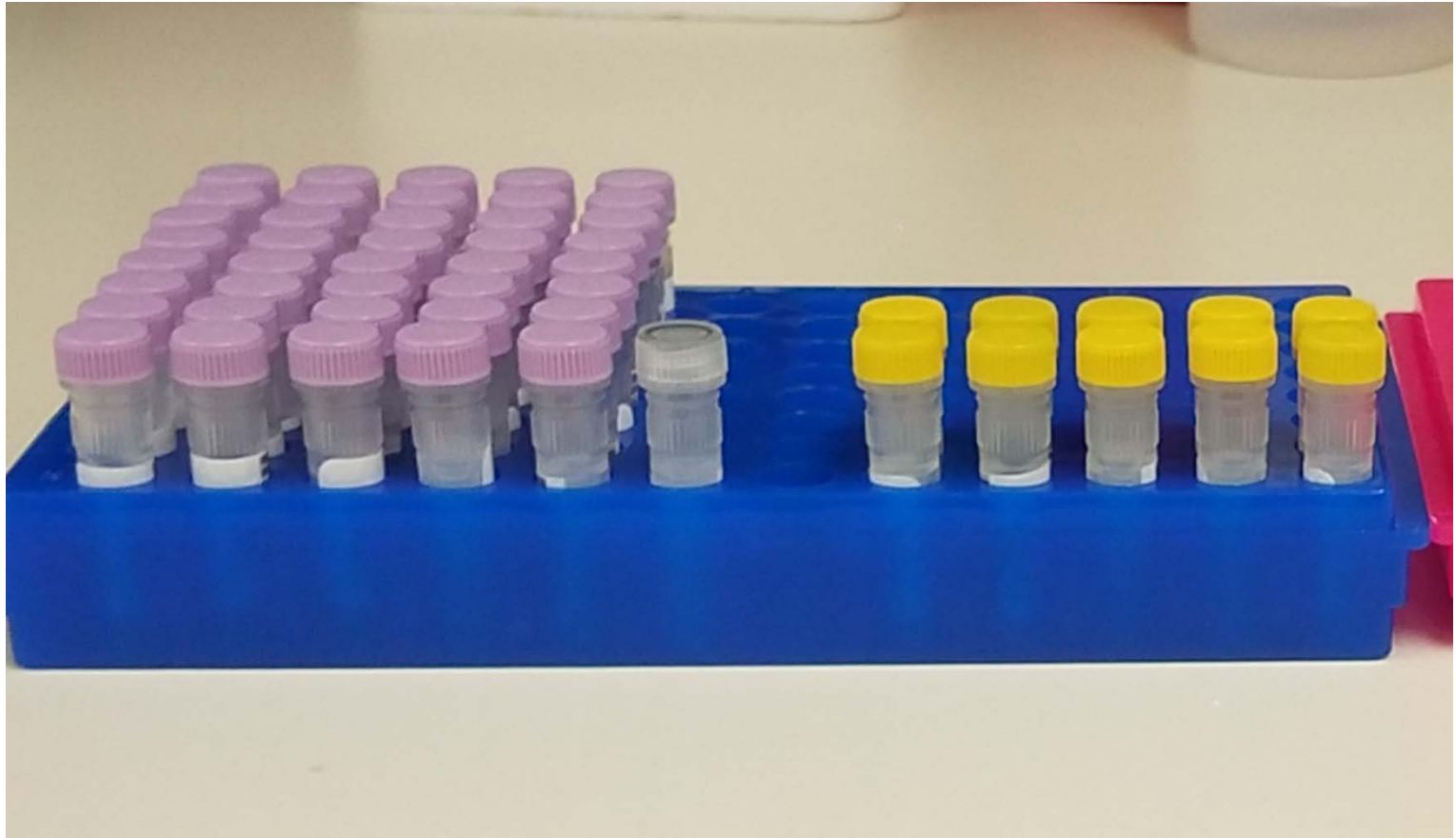
Slow decel on centrifuge makes it easier to pipet Buffy Coat



Pipet Buffy Coat using wide tip pipet, try to minimize RBC

Buffy Coat
1 aliquot

Venous Blood



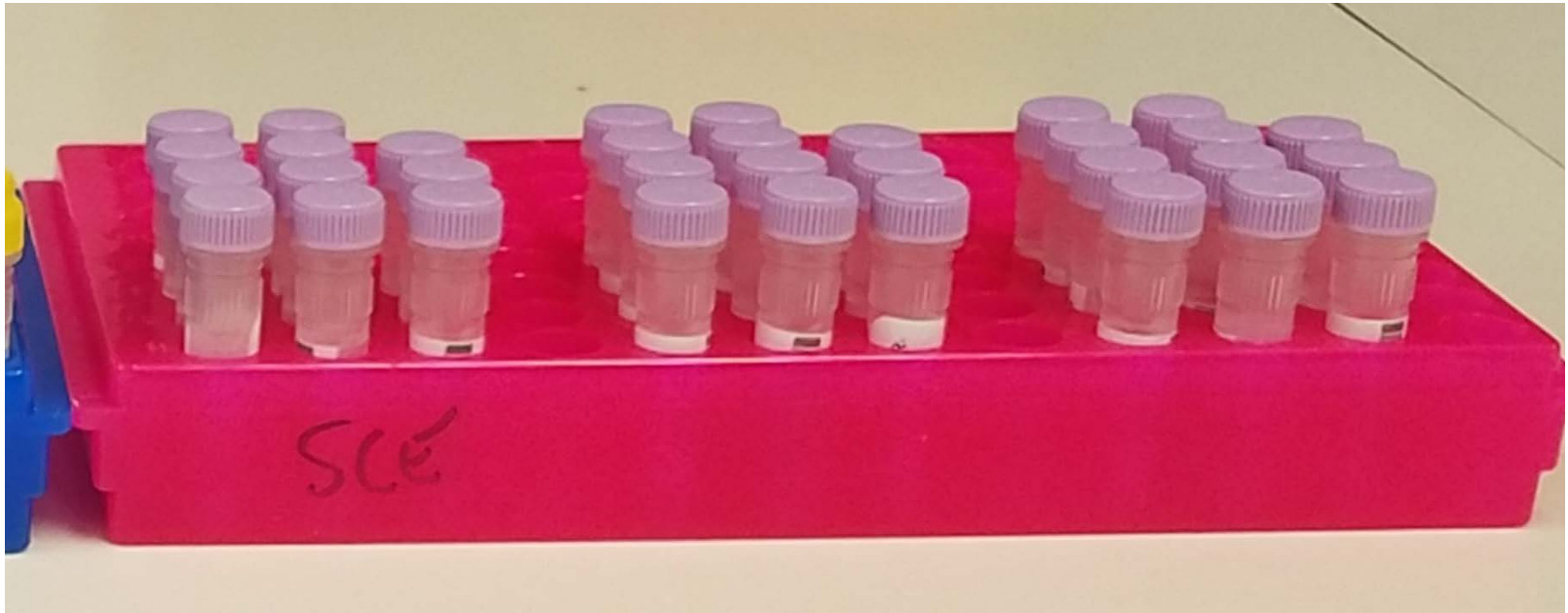
Plasma
40 aliquot
0.2ml into each tube

Buffy Coat
1 aliquot

Serum
10 aliquot
0.2ml into each tube

Place immediately
into dry ice

Mix, Wedge, and Syst Art Blood cyrovials



Plasma Mix
11 aliquot
0.2ml into each tube

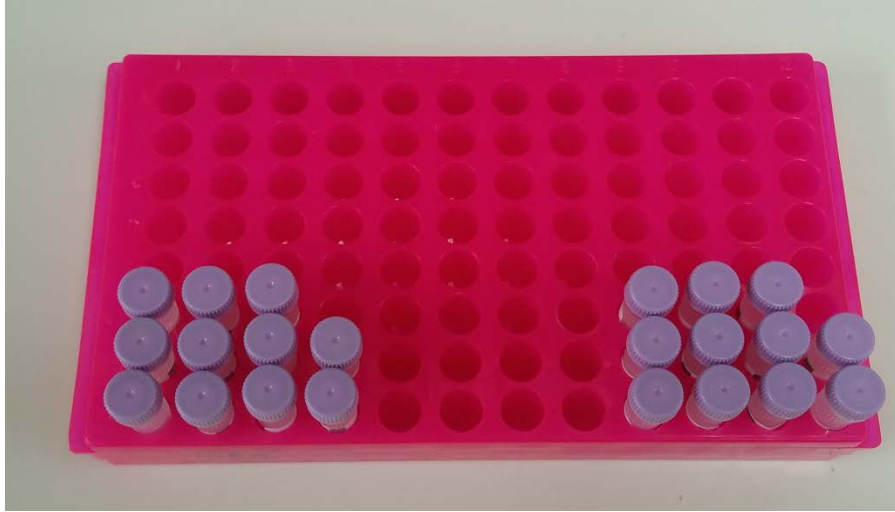
Plasma Wedge
11 aliquot
0.2ml into each tube

Plasma Syst Art*
11 aliquot
0.2ml into each tube

Place immediately
into dry ice

*Optional

Peak and Post Exercise cyrovials



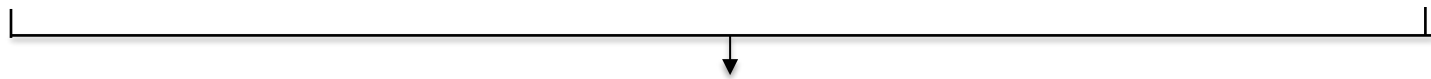
Peak Plasma Wedge
11 aliquot
0.2ml into each tube

Peak Plasma Syst Art
11 aliquot
0.2ml into each tube



Post Plasma Wedge
11 aliquot
0.2ml into each tube

Post Plasma Syst Art
11 aliquot
0.2ml into each tube



Place immediately
into dry ice

For Questions Please Contact

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