Colchester Research Facility
Laboratory for Clinical Biochemistry Research (LCBR)
University of Vermont
Department of Pathology and Laboratory Medicine

Lab Director: Russell P. Tracy, Ph.D.
Coordinator: Elaine Cornell
Lab/Project Manager: Rebekah Boyle, M.S.
Repository Manager: Sarah Nightingale

Presented by: Jessica Rooney

LCBR

- Russell P. Tracy, Ph.D has directed the LCBR since its inception in 1986; ABCC Board-certified Clinical Chemist
- Current Personnel includes 8 faculty members, 25 technical and administrative staff, plus students, post-doctoral fellows, and visiting scientists
- Focus on epidemiological & clinical trials research in the areas of coagulation, fibrinolysis, fibrosis, and innate and adaptive immunity
- Frequently serve as a central analysis laboratory and sample repository

Role of the LCBR in MESA 6

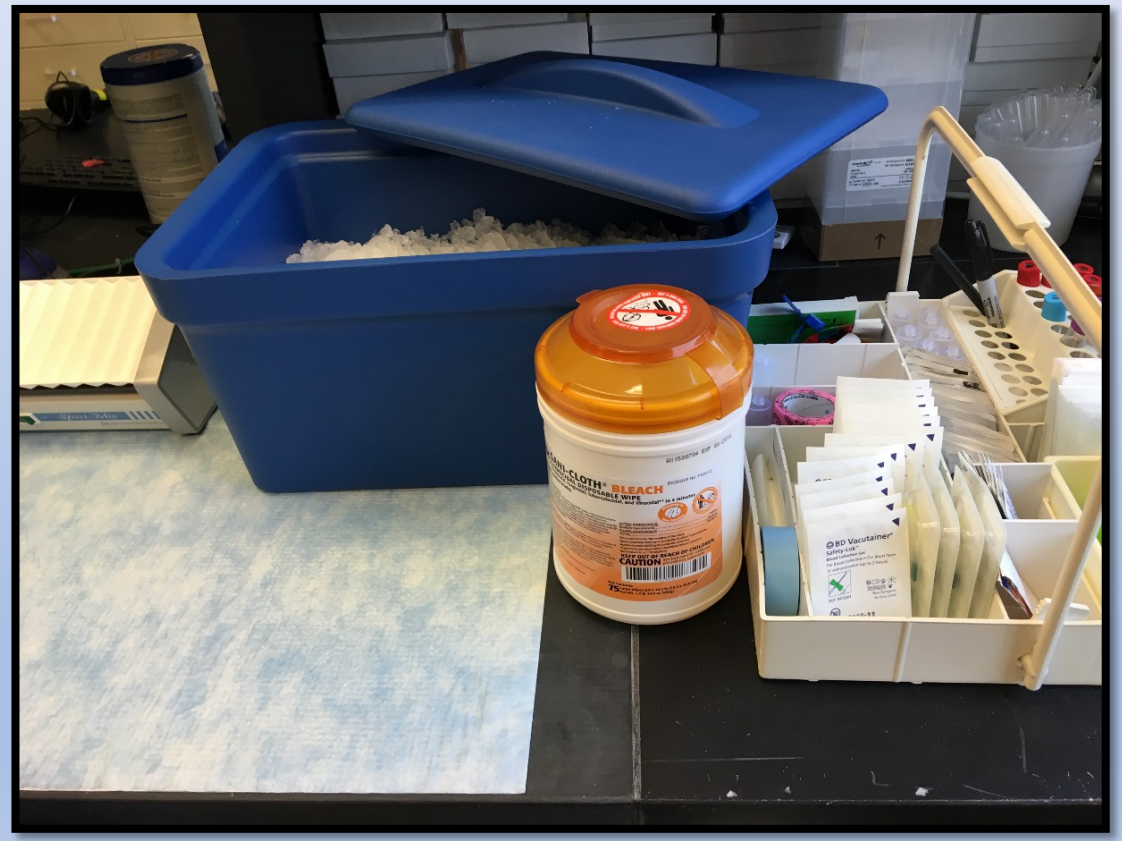
We are the MESA 6 Central Blood Analysis Lab (CBAL) and Repository

What we do:

- Assist with planning and development of MOPs for blood collection, handling, storage, sample utilization, etc.
- Receive shipments of participant samples from study sites
- Provide quality assurance feedback
- Perform some biomarker testing along with University of Minnesota
- Inventory and provide repository storage for study samples

Blood Collection

Set Up – Venipuncture Supplies



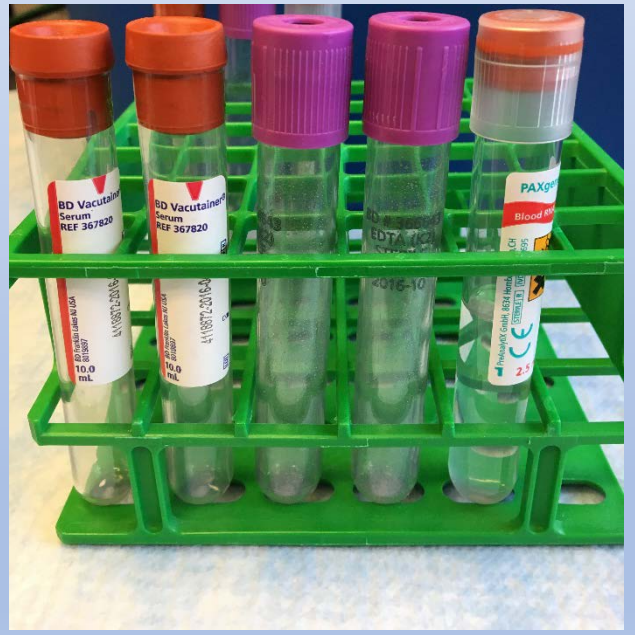
Draw Tubes

MESA 6 Classic Draw (All Study Sites)			
Quantity	Type	Volume	Total Volume
2	Serum	10 mL	20 mL
2	EDTA	10 mL	20 mL
1	PaxGene RNA	2.5 mL	2.5 mL
	Total Draw		42.5 mL

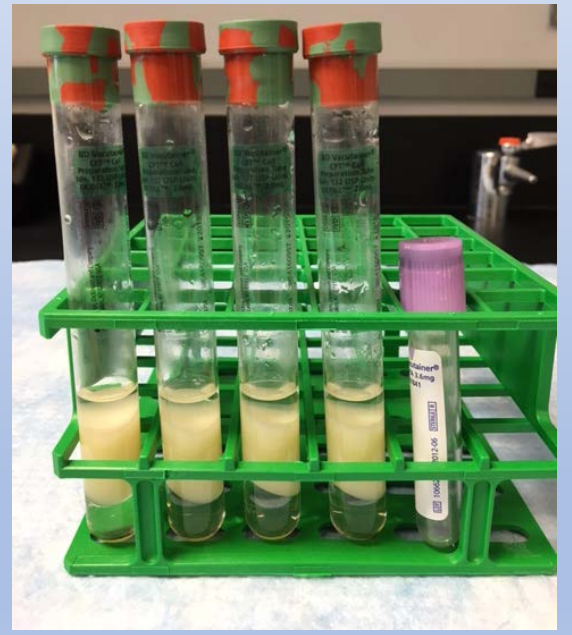
MESA Classic + Epigenomics (WFU, Minnesota, Columbia, JHU)			
Quantity	Type	Volume	Total Volume
2	Serum	10 mL	20 mL
2	EDTA	10 mL	20 mL
1	PaxGene RNA	2.5 mL	2.5 mL
4	CPT (Heparin)	8 mL	32 mL
1	EDTA	2 mL	2 mL
	Total Draw		76.5 mL

MESA Classic + NA Tissue (Northwestern)			
Quantity	Type	Volume	Total Volume
2	Serum	10 mL	20 mL
2	EDTA	10 mL	20 mL
1	PaxGene RNA	2.5 mL	2.5 mL
1	Serum	5 mL	5 mL
1	Heparin	10 mL	10 mL
	Total Draw		57.5

MESA 6 Classic Draw

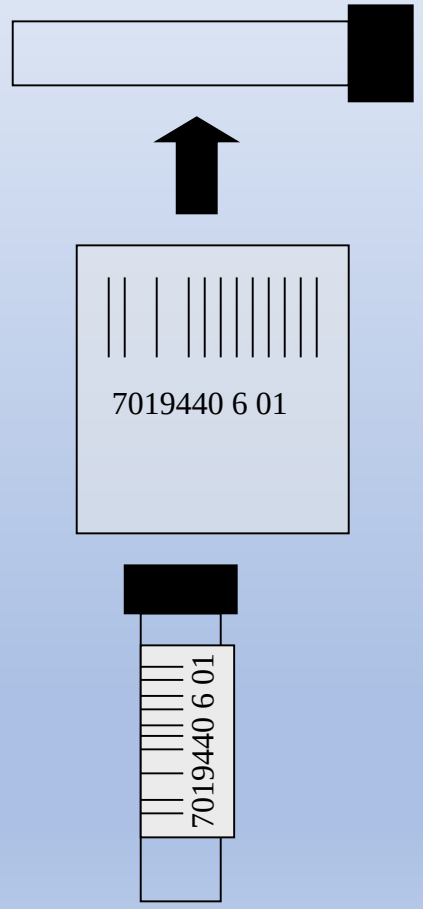


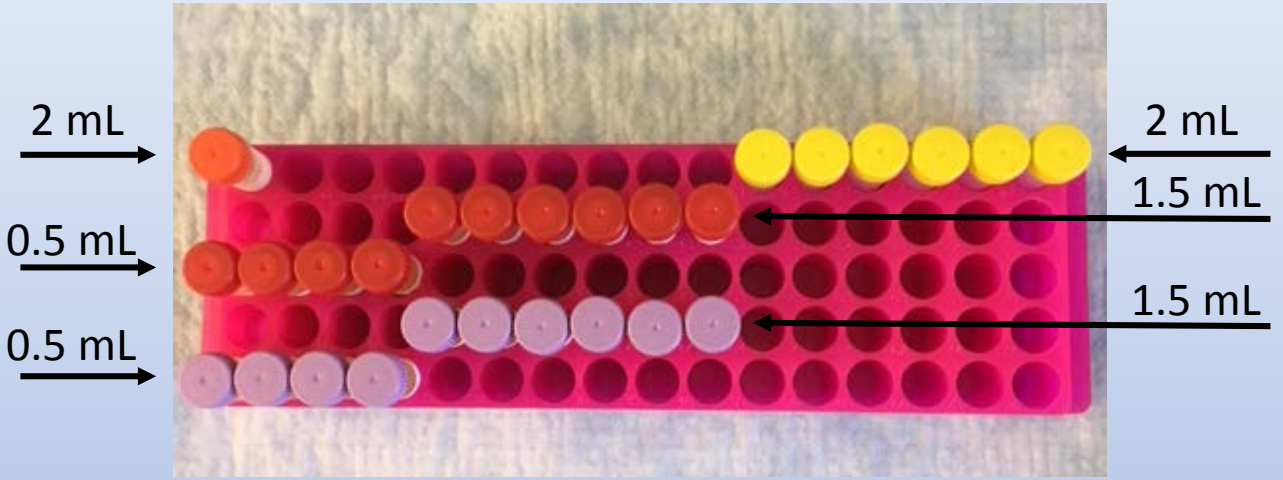
Additional tubes for Epigenomics

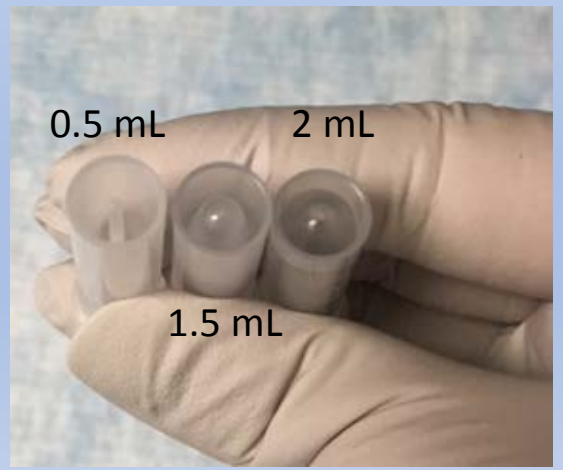


Additional tubes for NA Tissue

Aliquot Tube Preparation







Safety Issues and Precautions for Handling Blood Specimens

In accordance with the OSHA regulations on bloodborne pathogens, the LCBR recommends the following lab safety protocol for the field center laboratories:

- ✓ Use of non-permeable lab coats, latex gloves, and face shields 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.
- ✓ Use of aerosol containers in all centrifuges.
- ✓ Follow ‘Standard Precautions' when handling any blood products.
- ✓ Contaminated needles and sharps shall be immediately placed in a puncture-resistant, leak-proof 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 on file at the Clinical Center.
- ✓ Limit distractions - Noise, radios, phones, etc for your safety and the safety of those around you

Sources of safety information:

http://www.cdc.gov/niosh/topics/bbp/

http://www.osha.gov/SLTC/bloodbornepathogens/index.html

http://www.cdc.gov/elcosh/docs/d0300/d000378/d000378.pdf

Preparation of Venipuncture Site

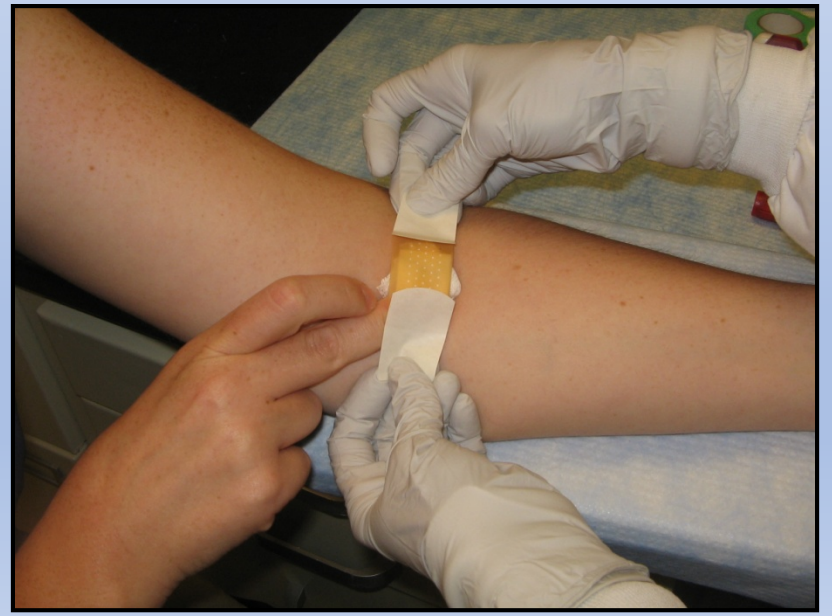
Venipuncture procedure

Venipuncture procedure continued

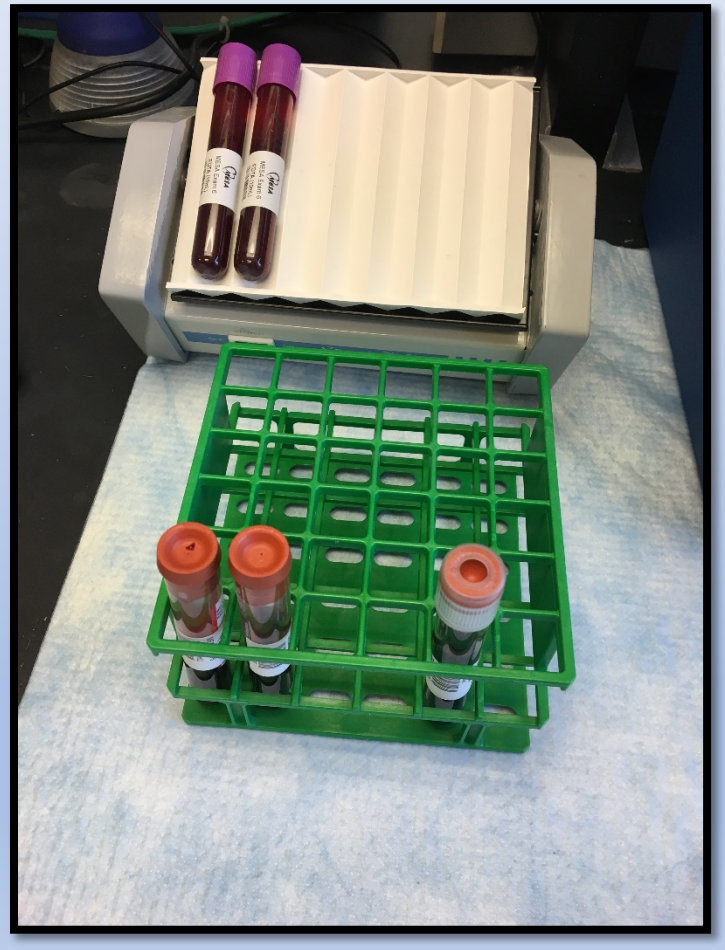
Venipuncture continued

← Remove needle and apply pressure w/ gauze pad

Clean venipuncture area; apply bandage →



MESA 6 Classic Samples after Draw

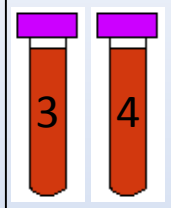


- Serum - After filling, gently invert tube 5 times to ensure proper mixing of the activator with the blood. Tubes remain upright at room temperature for a minimum of 60 minutes, but not longer than 90 minutes, to allow the blood to clot.
- EDTA - After filling, these tubes are mixed for ~30 seconds on the tube rocker then placed on wet ice for no more than 30 minutes before centrifugation.
- Paxgene - After filling, gently invert tube 8-10 times to mix blood with RNA stabilization additive. The tube can remain at room temperature until it is placed in a -80 C freezer for storage.

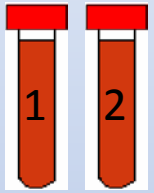
Blood Processing

MESA Exam 6 Processing Guide

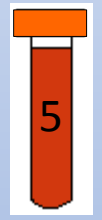
After the visit, you should have:



10 ml EDTA



10 ml SST



10 ml Paxgene

Plain Urine Cup

Remove 0.5 mL WB from EDTA tube #1 and place in 2 mL tube for HbA1c.

Place on wet ice until ready to centrifuge (<60 min)

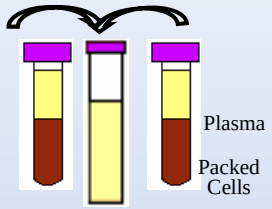
Let sit at room temp. for at least 60 minutes

CENTRIFUGE

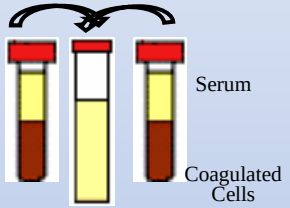
Do NOT Centrifuge (Leave at room temp. until ready to freeze)

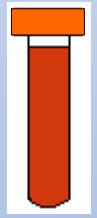
Aliquot immediately or place in refrigerator

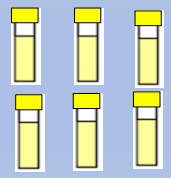
Pool Plasma from both EDTA Tubes



Pool Serum from both Serum Tubes







Cryovial #1 –0.5 mL whole blood in 2.0 mL cryo or HbA1c

From the pooled plasma tube pipette 0.5 mL plasma into Cryovials #2-5 and 1.0 ml in cryos 6-11

From the pooled serum tube pipette 0.5 ml serum into cryovials #12-15 and 1.0 ml into cryos 16-21

Place Paxgene (Tube 28) in larger freezer box

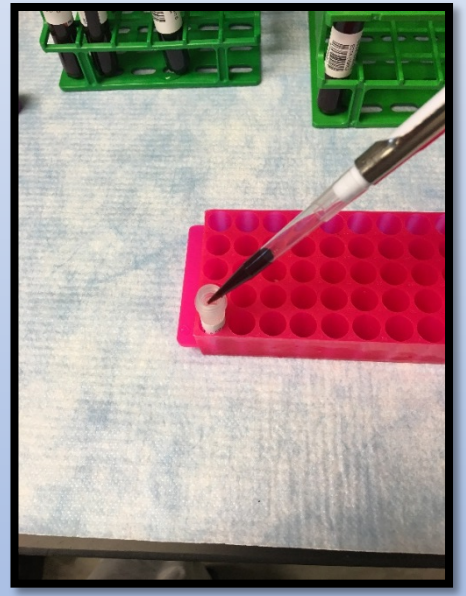
From the urine cup pipette 1.5 mL urine into 2.0 mL cryovials #22-27

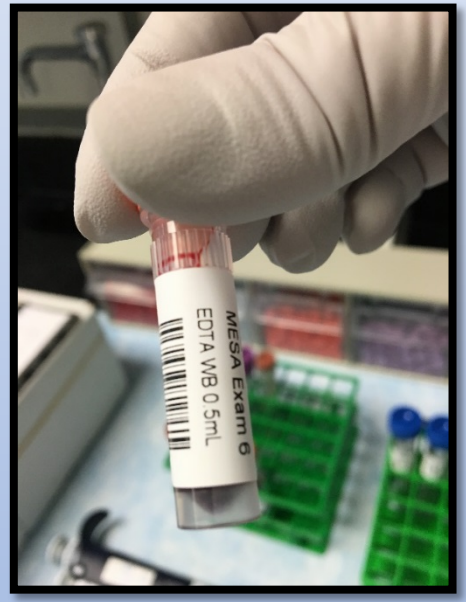
Freeze All Aliquots

Remove Whole Blood EDTA for HA1C

Before centrifuging EDTA tubes:

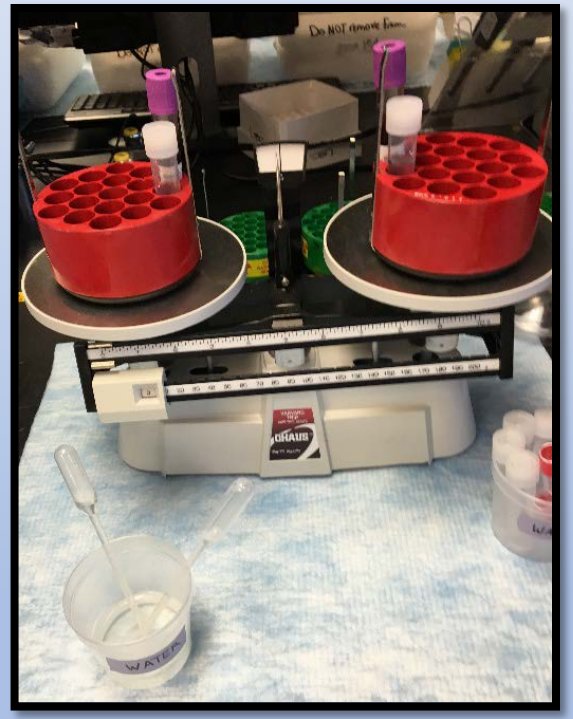
- Gently invert one EDTA draw tube to mix if sample has started to separate
- Carefully remove cap using absorbent pad to avoid splash/splatter
- Remove 0.5 mL of EDTA whole blood with pipette and place in pre-labeled 2 mL tube
- Cap with an orange cap
- Place on ice until sample can be frozen





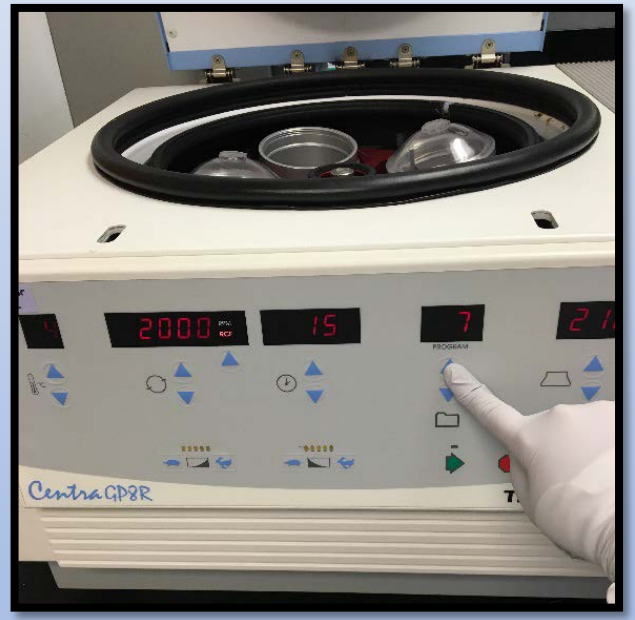
Prepare for Centrifugation

Balance tubes to be centrifuged



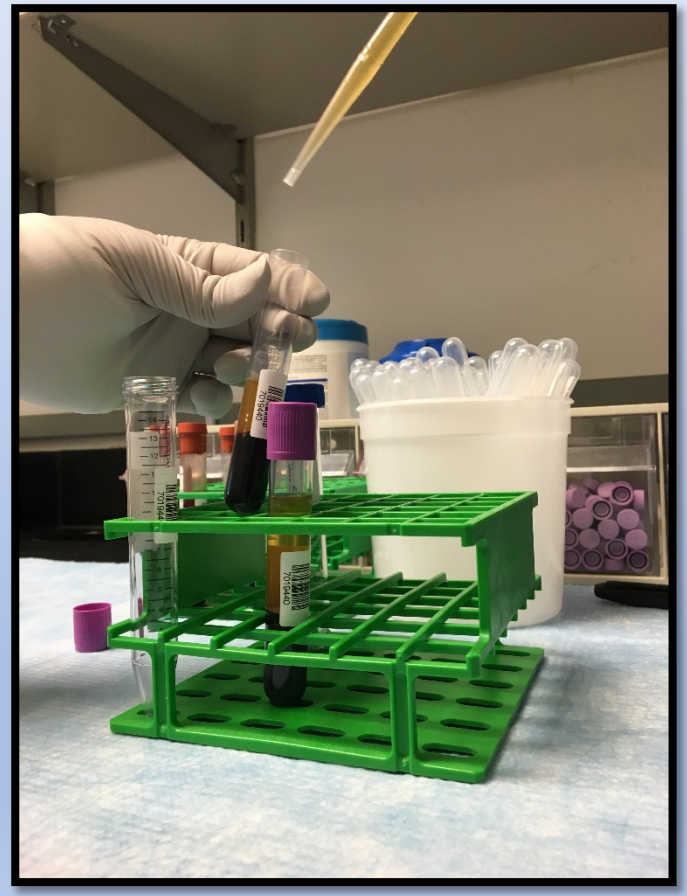
Use sealed cups to minimize aerosol hazard

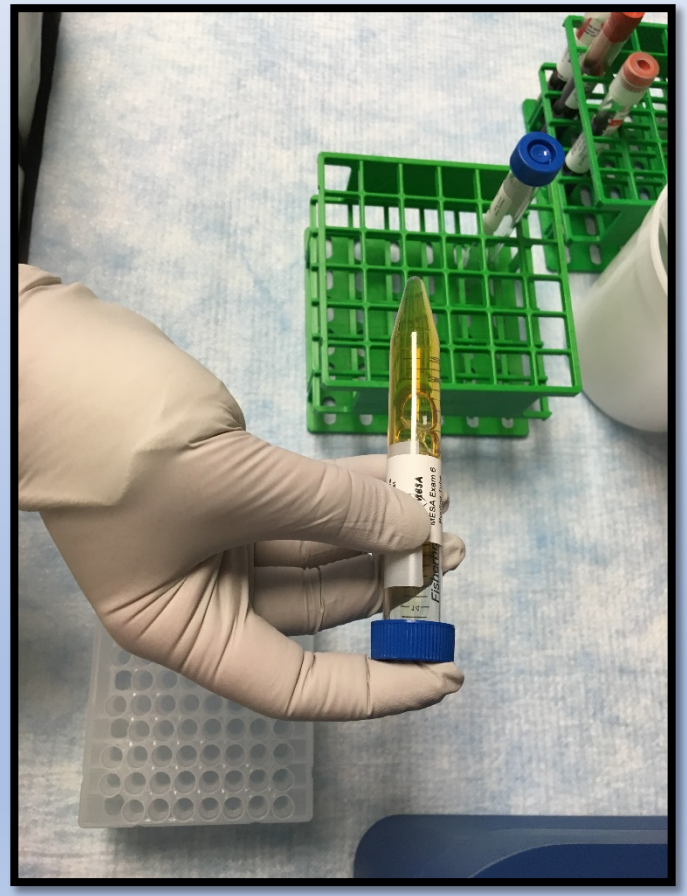
Run at 4°C for 30,000 G/min



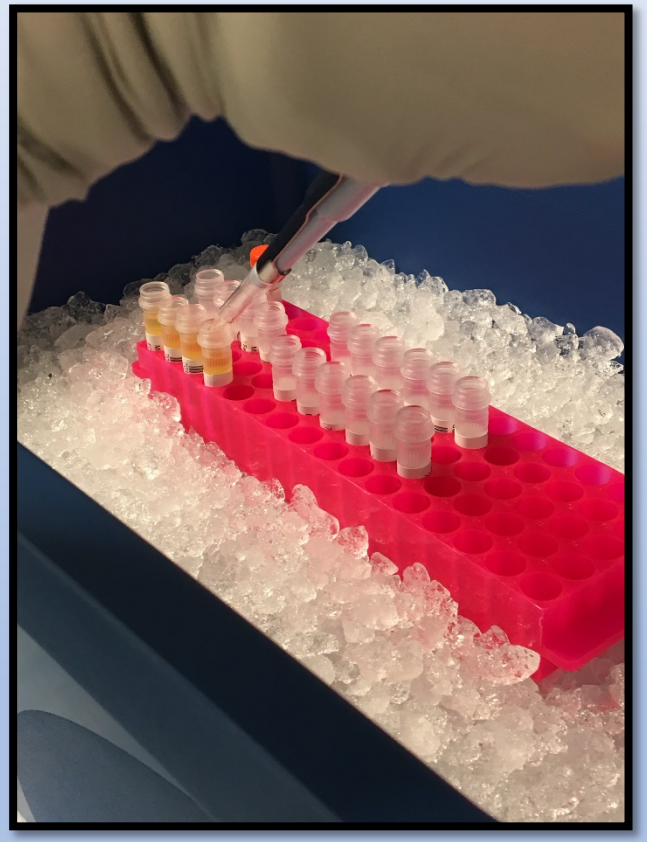
Pool and Gently Mix

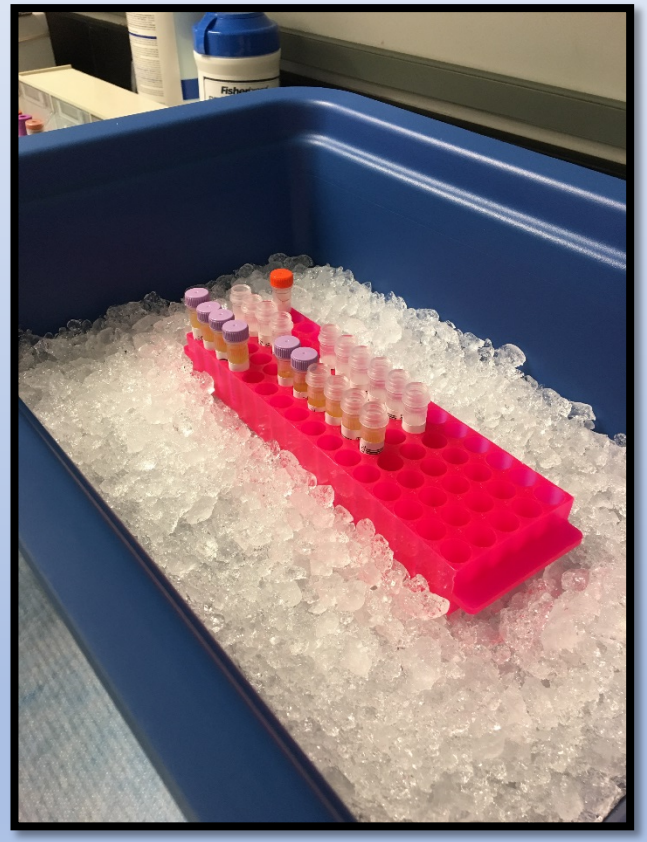
- Have labeled pooling tube ready
- Draw plasma off using a pipette
- Use care to avoid disturbing the cell layer at the bottom of the tube
- Cap and gently rock tube to mix





Aliquot and Cap

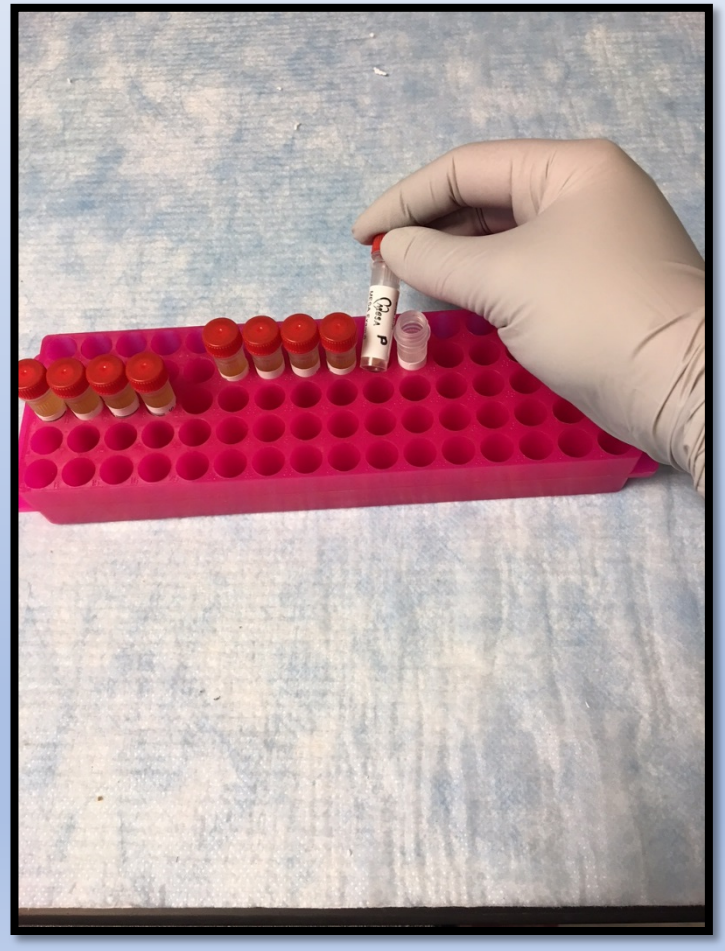




- Place rack with pre-labeled cryovials on ice or cold pack
- Aliquot 0.5 mL sample into first 4 cryos
- Aliquot 1 mL sample into remaining 6 cryos
- Cap full cryos with colored cap (EDTA-purple, serum-red, urine-yellow)
- Change pipette tips between sample types and participants

Processing Notes

- Serum sample processing follows the same steps as EDTA plasma
- Serum must sit for a minimum of 60 minutes before centrifugation to allow for blood to clot
- Fill tubes in numerical order
- Do not attempt to make all aliquots if only a partial volume was collected, fill cryos in order with specified volume leaving the last with partial volume
- Mark partial volume samples on the label with a “P” and record on the processing form
- Record all aliquots made and any deviations from protocol on processing form



Urine Collection and Processing

‘spot’ urine collection
~11 mLs required for processing

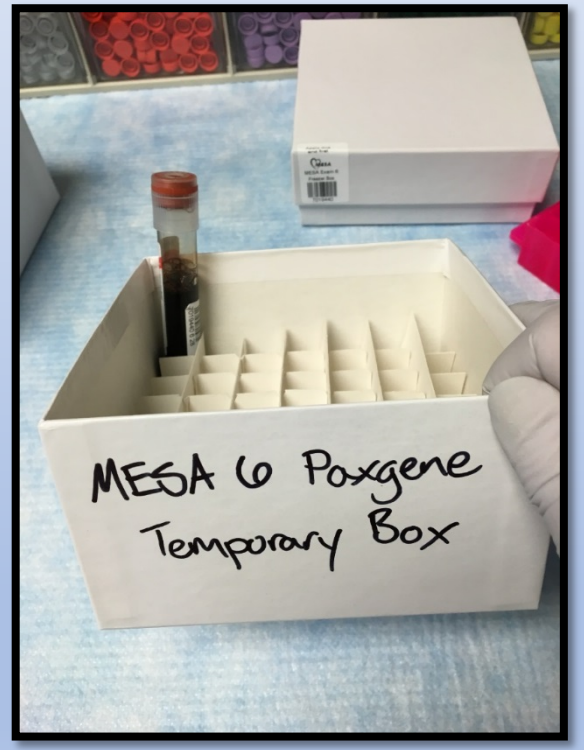
1.5 mL x 6 cryos

Labeled urine aliquots

- Cryovials 22-27, 2 mL size vial
- 1.5 mL urine added to each tube
- Place all tubes on ice until the can be frozen @ -80 C

(preferably within 10 mins)

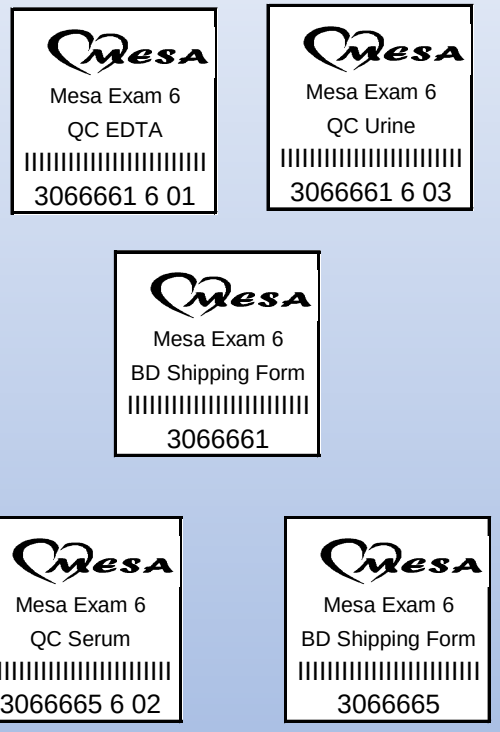
Prepare for Freezer

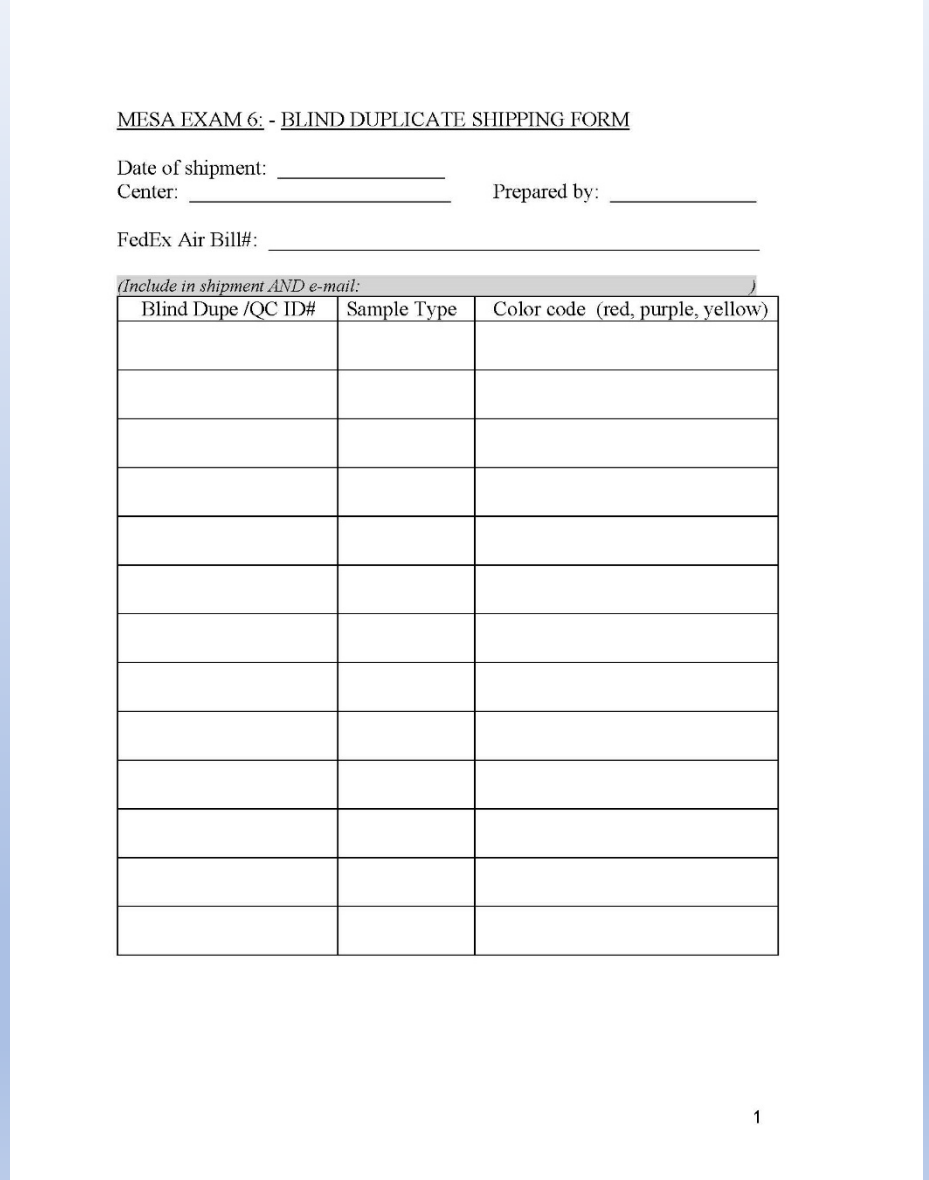


- Place all cryovials in 5x5x2 inch fiberboard box with 81 cell grid
- Label box with “Freezer Box” label
- Place Paxgene RNA draw tube in a temporary box or rack to freeze upright
- All samples should be frozen upright in a -80^{o}C freezer until ready to ship

Blind Duplicates for QC

- A blind duplicate sample for quality control will be reserved from ~10% of participants (5% each for EDTA and serum.).
- Participants selected for EDTA QC activity will automatically be selected for urine QC.
- Participants will be selected for QC purposes based on the last two digits of their ID numbers.
- EDTA & urine QC activity last 2 digits of their 7 digit MESA ID are 06, 16, 26, 36, 46, 15.
- Serum QC activity last 2 digits of their 7 digit MESA ID are 56, 66, 76, 86, 96, 75.





MESA EXAM 6: - BLIND DUPLICATE SHIPPING FORM

Date of shipment: _____
Center: _____ Prepared by: _____

FedEx Air Bill#: _____

(Include in shipment AND e-mail:)

Blind Dupe /QC ID#	Sample Type	Color code (red, purple, yellow)

1

Packing and Shipping

Participant Blood Sample Freezer Box Diagram

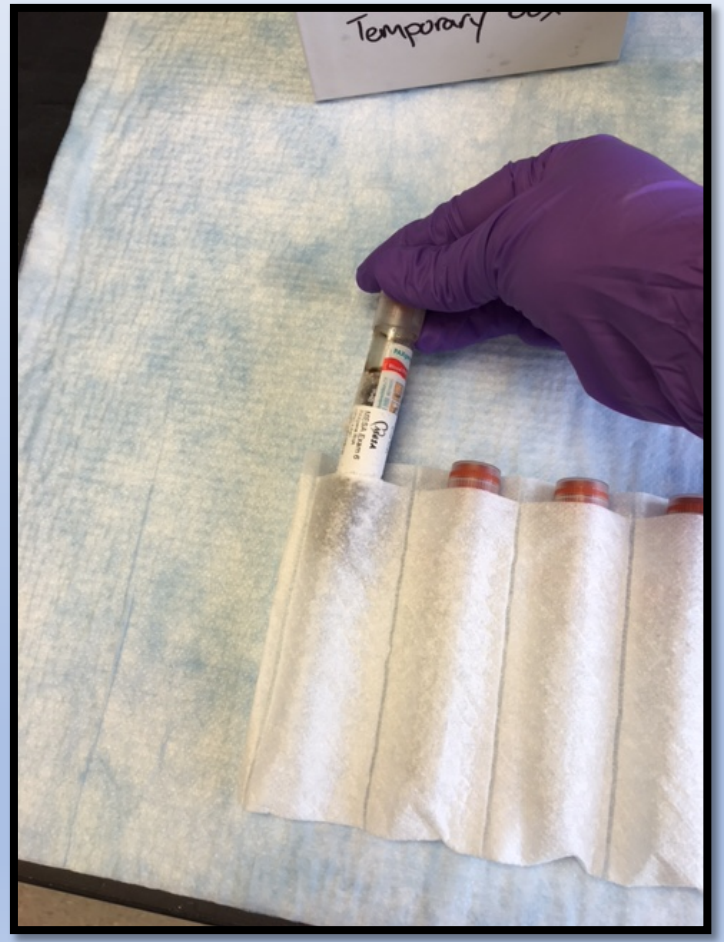
PT1 Cryo 1	Cryo 10	Cryo 19	**PT2** Cryo 1	Cryo 10	Cryo 19	**PT3** Cryo 1	Cryo 10	Cryo 19
Cryo 2	Cryo 11	Cryo 20	Cryo 2	Cryo 11	Cryo 20	Cryo 2	Cryo 11	Cryo 20
Cryo 3	Cryo 12	Cryo 21	Cryo 3	Cryo 12	Cryo 21	Cryo 3	Cryo 12	Cryo 21
Cryo 4	Cryo 13	Cryo 22	Cryo 4	Cryo 13	Cryo 22	Cryo 4	Cryo 13	Cryo 22
Cryo 5	Cryo 14	Cryo 23	Cryo 5	Cryo 14	Cryo 23	Cryo 5	Cryo 14	Cryo 23
Cryo 6	Cryo 15	Cryo 24	Cryo 6	Cryo 15	Cryo 24	Cryo 6	Cryo 15	Cryo 24
Cryo 7	Cryo 16	Cryo 25	Cryo 7	Cryo 16	Cryo 25	Cryo 7	Cryo 16	Cryo 25
Cryo 8	Cryo 17	Cryo 26	Cryo 8	Cryo 17	Cryo 26	Cryo 8	Cryo 17	Cryo 26
Cryo 9	Cryo 18	Cryo 27	Cryo 9	Cryo 18	Cryo 27	Cryo 9	Cryo 18	Cryo 27

Freezer Box design for shipping FROZEN samples to CBAL

- Samples are shipped frozen on Dry Ice to Vermont every other week.
- Cryovials #1-27. Three participants per freezer box.

Shipping Frozen Paxgene Samples

- Remove samples to be shipped from freezer
- Ensure tubes remain frozen during packing
- Place individual Paxgene tubes in absorbent tube sleeves
- Lay tubes in absorbent sleeves in box
- If there are 12 or less tubes use a 5x5x2 inch box
- If there are greater than 12 tubes use a 5x5x3 inch box
- Secure box with rubberband



Packing Frozen Blood Samples

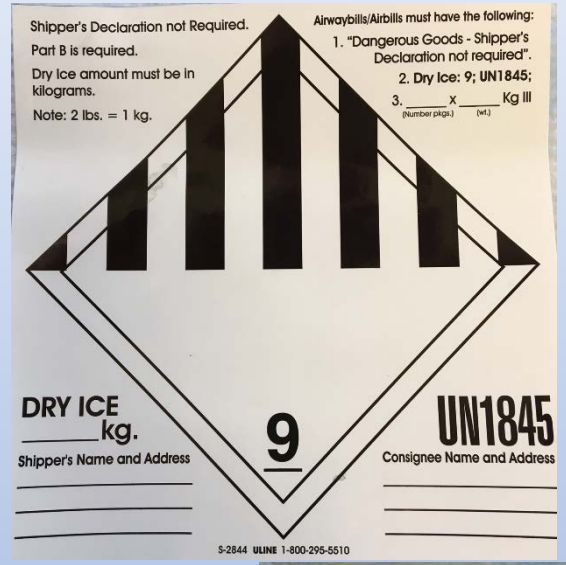
The following shipping instructions comply with the International Air Transport Association's Goods Regulation-Packaging Instructions 650 and 954

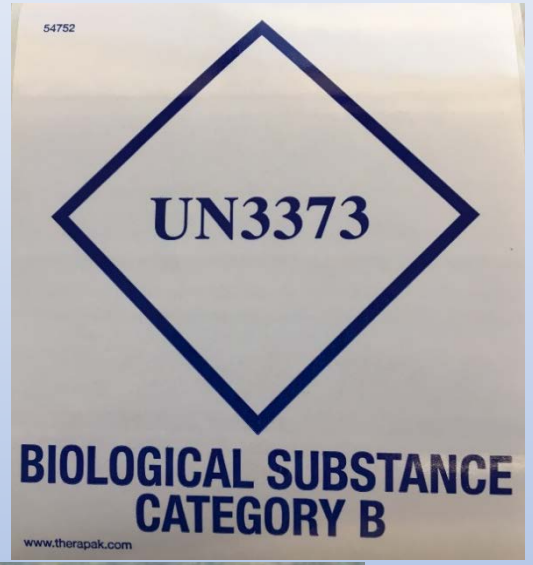
- Line Styrofoam mailer with cushioning/absorbent material (lab mat)
- Place ~5lbs of dry ice in bottom of mailer
- Place another layer of absorbent material on top of dry ice to act as a barrier between the dry ice and freezer boxes
- Ensure cryovials are packed in 5x5x2 inch cardboard freezer boxes with either 81 or 100 cell grids
- Secure boxes with rubber bands and place in a zip top bag with absorbent material sufficient for the volume of sample being sent
- Ensure Paxgene tubes are packaged according to instructions and placed in zip top bag
- Place bagged samples into Styrofoam mailer
- Place another layer of absorbent material over freezer boxes
- Place remaining dry ice (~5 lbs) on top of absorbent material and put the top of the Styrofoam in place
- Insert the inventory manifest and participant forms on top of the Styrofoam mailer before sealing the outer cardboard flaps

Shipping Schedule

- Frozen samples should be shipped Monday or Tuesday of every other week
- We would like to develop a shipping schedule so every site ships the same week, but are divided between the two days
- Please let us know if you have any limitation at your site that are related to schedule (Dry Ice availability, etc)

Shipping Frozen Blood Samples





- Use Fed-Ex Priority Overnight Delivery
- Shipment information should be e-mailed to Elaine Cornell and Sarah Nightingale(elaine.cornell@med.uvm.edu & sarah.nightingale@med.uvm.edu)
- All packages must be marked with
  - A UN3373 Biological Substance Category B label
  - A dangerous goods Category 9 UN1845 Dry Ice label (with dry ice weight)
  - A completed Fed-Ex air bill
  - The shipper and recipient's name, address, and phone number
- All packages should be shipped to:

  Rebekah Boyle
  University of Vermont
  Laboratory for Clinical Biochemistry Research
  360 South Park Drive
  Colchester, VT 05468
  p:(802)656-8938

Shipping Heparin Samples (NWU Only)

Pending

All packages should be shipped to:

Peggy Doyle
University of Vermont
Laboratory for Clinical Biochemistry Research
360 South Park Drive
Colchester, VT 05468
p:(802) 656-8939

Certification

Certification Process

Obtaining Certification

- Read and understand the appropriate chapters in the MESA 6 Manual of Operations.
- Observe the process performed by a certified technician
- Successfully complete the written exam
- Successfully complete the practical exam

Maintaining Certification

- Perform phlebotomy/processing on a minimum of one person every month for the first three month and every other month after that

Certifying Other Technicians

- Ensure new technician has read MESA's Laboratory MOP and section of the Exam 6 MOP.
- Perform phlebotomy/processing with new technician observing
- Supervise as new technician processes blood samples from one volunteer as described in the protocol and in accordance with the certification checklist
- Administer written examination prepared by LCBR.
- Send exams and checklists to LCBR and CC


Questions?