1.0 Appendix A: Pancreas Blood SOP

Revised 11/21/2011

Appendix A

NOTE, ALL SPECIMENS SHOULD BE FROZEN BELOW –70ºC NO LONGER THAN 4HRS AFTER BLOOD DRAW.

Principle:

The collection of human blood samples for proteomic analysis requires that patient sample collection, storage, transport, and handling remain consistent within rigid guidelines for optimal results

Required Materials:

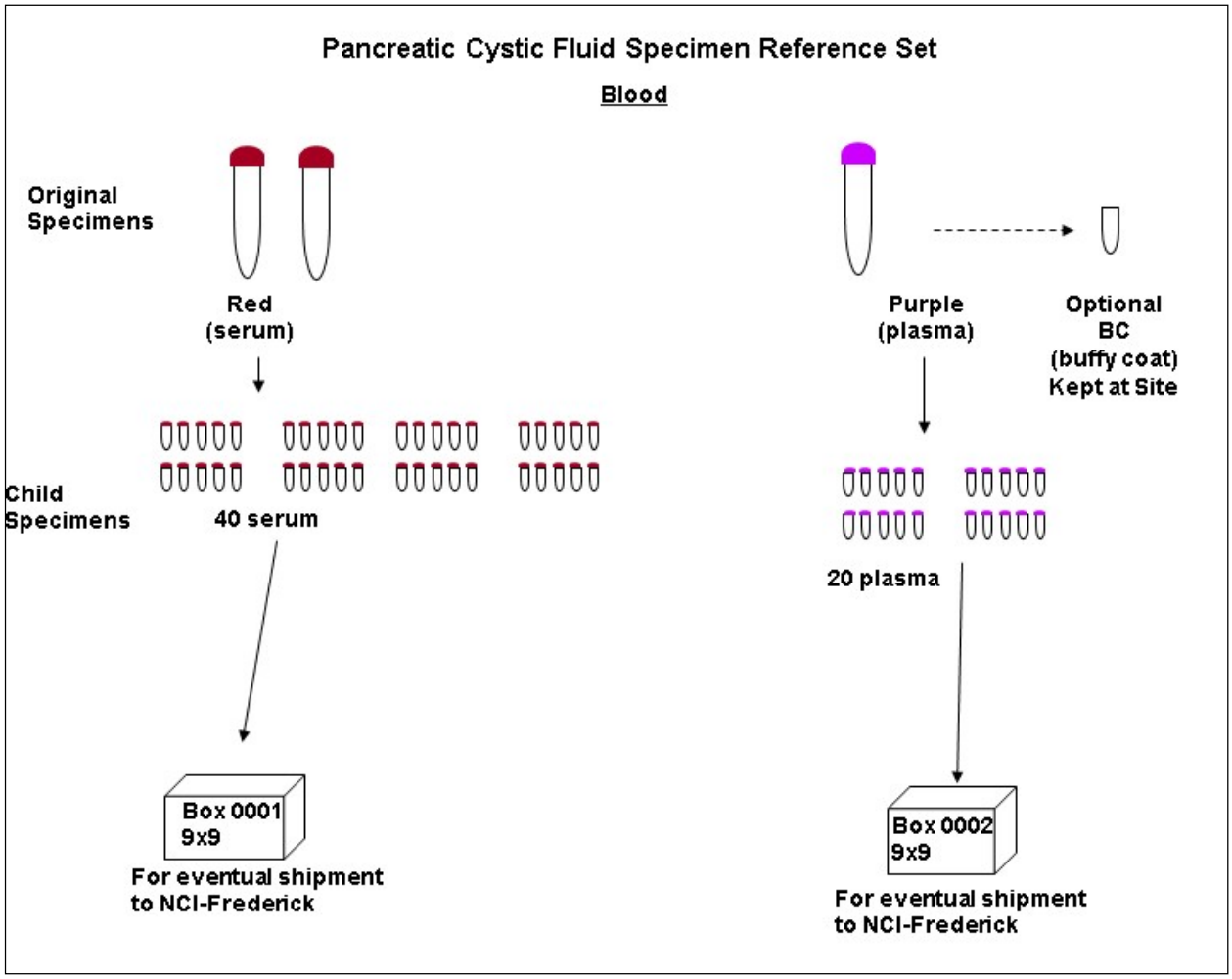
1. Vacutainer needles, 21G ¾ x 12" Safety-Lok Blood Collection Set , and vacationer hub or Butterfly needle, attached tubing and Luer adapter
2. Serum: Two 10ml red top tubes, non-additive (BD366430) http://catalog.bd.com/bdCat/viewProduct.doCustomer?productNumber=366430
3. Plasma (EDTA): One 10ml EDTA tubes Plasma-EDTA: One 10 ml (draw) EDTA plastic tube (BD366643) http://catalog.bd.com/bdCat/viewProduct.doCustomer?productNumber=366643
4. Aliquot containers for all blood specimens (20 blue cap tubes for plasma and 40 red cap tubes for serum) -.5 ml Polypropylene Micro Tubes, screw top, conical skirted (Sarstedt 72.730) http://www.sarstedt.com/php/main.php
5. Lab equipment: 1000 µl micropipettor, tips, transfer pipettes, 2x 15mL conical tube
6. Aliquot Inventory Sheet /Collection Sheet (bar-coded sheet)
7. Shipping materials (reusable)
8. Tourniquets (non-latex)
9. Alcohol prep pads
10. Bandages
11. Sterile gauze pads
12. Centrifuge (refrigerated or non-refrigerated), swinging bucket rotor with adjustable brake and speed (1200-1500xg) with lids
13. Lab supplies: bleach, sharps container, gloves, Personal Protective Equipment (PPE), face mask, and goggles
14. Ice for processing plasma and serum samples
15. Ultra-cold freezer (-70°C or colder)
16. Dry ice for monthly shipments
17. 2-D Bar code scanner

1.1 Specimens collection and sample management:

- The specimen of choice for proteomic analysis is serum obtained from whole blood collected in red top vacutainer tubes with no additives or clot activators
- Order of draw – First, 2 x non-additive tubes (glass, red stopper) Second, 1x EDTA tubes (plastic, lavender stopper). Multiple blood draws may be performed to obtain necessary volume and/or to ensure that specimens are collected within 12 weeks prior to Surgery (if performed) or EUS.
- The red top non-additive blood specimens should be allowed to clot for **40 – 50 minutes** with tube **upright** at **room temperature**
- The lavender EDTA tubes should be filled to line on label for proper EDTA blood ratios, inverted 4--6 times to mix, then placed flat on ice until processed. (Wait for tube to cool down before introducing it to ice)

- Transport blood in cooler labeled with a Biohazard sticker. Make sure there is an ice pack or ice available for refrigeration when necessary
- All centrifugation steps should be at room temperature
- After centrifugation the plasma and serum should be aliquoted into bar-coded Polypropylene Micro Tubes and frozen at -70°C or colder **within 4 hours of collection**
- The serum should be free of hemolysis and clots by visual inspection. Hemolysis is defined as any plasma or serum sample that is reddish in color

Figure 1- Flow Chart



1.2 Procedure:

1. Locate 2 Red Top Tubes, 1 EDTA Tube, the Specimen Collection Worksheets with corresponding labels and all other blood drawing supplies (Please check to make sure that the blood tubes are not expired).
2. Information regarding fasting status should be recorded on Specimen Collection Worksheet at time of blood draw
3. Assemble the supplies to be used in obtaining the specimen. Label the red and lavender top blood tubes with Kit# labels once specimen is obtained. Document the time of draw on the specimen collection worksheet.

4. Put on disposable gloves. 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.
5. 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.
6. Clean the forearm of the patient with antiseptic wipe in a circular motion beginning at the insertion site. Allow the antiseptic to dry.
7. 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.
8. Using the dominant hand, insert either the vacutainer needle or the butterfly needle (if using vacutainer needle, attach hub first.) Push the evacuated tube onto the vacutainer hub or the Luer adapter if using a butterfly.
9. Release the tourniquet once blood flow is established.
10. 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.
11. Lightly place a sterile gauze pad over the venipuncture site. Gently remove the needle.
12. Apply pressure to the site with sterile gauze. Apply bandage. Instruct the patient to leave the bandage on for at least 15 minutes.
13. Dispose of the needle in a sharp's container.
14. Remove gloves and wash hands.
15. Associate Kit# to Participant ID in VSIMS

1.3 **Serum (Red stopper):**

1. Affix labels to the red cap aliquot tubes in numerical order (ex: SR01, SR02, SR03 etc.).
2. Leave red top blood tubes upright at room temperature for at 40-50 minutes (this allows the clot to form). Tubes can be refrigerated after until centrifugation.
3. Balance red-top tubes opposite each other in centrifuge. Take buckets off centrifuge and balance on a scale if necessary. Make sure lids are on buckets when spinning.
4. Centrifuge at 1200xg for 10 minutes at room temperature. (This can be done at the same time as the plasma spin with the slower brake.)
5. Aliquot 100uLs of serum (the supernatant or upper layer) from both tubes into each of the 40 Polypropylene Micro Tubes with red caps

Use all serum except the last 1/4 inch to avoid red blood cell contamination. If a gel-like mass is present, pierce gently with a pipette tip and re-centrifuge at 1200xg for 5 minutes, then aliquot into desired amount. Serum should be free of hemolysis (reddish in color). If the sample is hemolyzed (reddish in color), aliquot the non-hemolyzed sample first, and then finish with the hemolyzed sample. **Any remaining serum may be kept at the site in their own storage, using their own standard way of labeling and storing.**

6. Freeze serum specimens in a –70ºC or colder freezer no more than 4 hours after the blood draw. Serum aliquots can be temporarily stored on wet ice until able to freeze at –70ºC or colder.

1.4 Plasma

1. Affix labels to the blue cap aliquot tubes in numerical order (ex: PL01, PL02, PL03 etc.).
2. Mix blood thoroughly after draw and leave lavender-top blood tube horizontal on ice until ready to process.
3. Balance lavender -top tube opposite a balancing tube in centrifuge making sure that opposite buckets are balanced (use a scale if necessary).
4. Centrifuge at 1200xg for 10 minutes with slowest brake. This spin can be performed at the same time as step 4 for processing the serums. (The slow brake enables the buffy coat to separate for easier processing). After centrifugation, the sample should separate into 3 layers: top layer is the plasma, middle thin white layer is the buffy coat and the bottom layer is the red blood cells. Follow both set of instructions for PLASMA and BUFFY COAT.
5. The resultant plasma (assume 40% yield) is transferred into appropriately non-labeled 15mL conical tube with transfer pipet. Leave 1/4 inch to prevent disturbing the buffy coat. Do not disrupt the white buffy coat layer.
6. The secondary tube is then centrifuged at 1500xg for 5 minutes (normal speed & brake) to remove any remaining cells and platelets.
7. Aliquot 100uLs of plasma (the supernatant or upper layer into each of the 20 Polypropylene Micro Tubes with blue caps.

Use all plasma except the last 1/4 inch to avoid cell contamination. Plasma should be free of hemolysis (reddish in color). If the sample is hemolyzed (reddish in color), aliquot the non-hemolyzed sample first, and then finish with the hemolyzed sample. **Any remaining plasma may be kept at the site in their own storage tubes, using their own standard way of labeling and storing.**

8. Freeze plasma specimens in a –70ºC or colder freezer no more than 4 hours after the blood draw. Plasma aliquots can be temporarily stored on wet ice until able to freeze at –70ºC or colder.

1.5 BUFFY COAT:

1. Using a transfer pipet, transfer the entire white buffy coat into a 15mL conical tube labeled with **your own standard way of labeling and storing**. To get the entire buffy coat, **you will need to take the remaining plasma and the top quarter of an inch of red blood cells**. This sample is not part of the reference set and is optional for the site. The sample will not be tracked on a worksheet, entered into VSIMS, or shipped to NCI-Frederick.
2. Store on wet ice until able to freeze below –70ºC or colder

1.5.1 Clean-up:

1. Replace stoppers and dispose of blood tubes in the plastic sharps bucket labeled as containing biohazardous material. Dispose of contaminated gloves, towels, and other non-sharps in biohazard waste. Soak anything that comes in direct contact with blood/plasma/serum in 10% bleach for at least 10 minutes before disposal in biohazard waste.
2. After aliquoting plasma, add 10% bleach to the conical tubes and leave for at least 10min before discarding. Bleach can then be discarded in the sink and the tubes can be discarded in the biohazard waste.

Example Worksheets:

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Kit 8	Participant ID (affix label):

(extra copies of this section provided to allow for multiple collection dates)

Staff ID: ____ Participant fasting (6-8 hrs of no food): ☐ Yes ☐ No ☐ Unknown

Date collected: __ - __ - ____ Time collected: __ : __ ☐ AM ☐ PM

Time at RT to clot (40-50 min): __ minutes

Time centrifuge started (10 min at 1200g): __ : __ ☐ AM ☐ PM (Exact time)

Time placed in freezer (within 15 minutes): __ : __ ☐ AM ☐ PM (Exact time)

Freezer temperature -70C or colder: ☐ Yes ☐ No

☐ 1st Red Top Tube collected ☐ 2nd Red Top Tube collected

☒ *if collected*	Volume	Freezer #	9x9 Box #	Row	Column	Is the specimen hemolyzed or lipemic or icteric? (circle all that apply)	SOP followed?
☐ SR01 3421009811	100 ul					H, L, I	Y / N / Unk
☐ SR02 3421009820	100 ul					H, L, I	Y / N / Unk
☐ SR03 3421009839	100 ul					H, L, I	Y / N / Unk
☐ SR04 3421009848	100 ul					H, L, I	Y / N / Unk
☐ SR05 3421009857	100 ul					H, L, I	Y / N / Unk
☐ SR06 3421009866	100 ul					H, L, I	Y / N / Unk
☐ SR07 3421009875	100 ul					H, L, I	Y / N / Unk
☐ SR08 3421009884	100 ul					H, L, I	Y / N / Unk
☐ SR09 3421009893	100 ul					H, L, I	Y / N / Unk
☐ SR10 3421009900	100 ul					H, L, I	Y / N / Unk
☐ SR11 3421009919	100 ul					H, L, I	Y / N / Unk
☐ SR12 3421009928	100 ul					H, L, I	Y / N / Unk
☐ SR13 3421009937	100 ul					H, L, I	Y / N / Unk
☐ SR14 3421009946	100 ul					H, L, I	Y / N / Unk
☐ SR15 3421009955	100 ul					H, L, I	Y / N / Unk
☐ SR16 3421009964	100 ul					H, L, I	Y / N / Unk
☐ SR17 3421009973	100 ul					H, L, I	Y / N / Unk
☐ SR18 3421009982	100 ul					H, L, I	Y / N / Unk
☐ SR19 3421009991	100 ul					H, L, I	Y / N / Unk
☐ SR20 3421010006	100 ul					H, L, I	Y / N / Unk

Kit 8	Participant ID (affix label):

☒ *if collected*	Volume	Freezer #	9x9 Box #	Row	Column	Is the specimen hemolyzed or lipemic or icteric? (circle all that apply)	SOP followed?
☐ SR21 3421010015	100 ul					H, L, I	Y / N / Unk
☐ SR22 3421010024	100 ul					H, L, I	Y / N / Unk
☐ SR23 3421010033	100 ul					H, L, I	Y / N / Unk
☐ SR24 3421010042	100 ul					H, L, I	Y / N / Unk
☐ SR25 3421010051	100 ul					H, L, I	Y / N / Unk
☐ SR26 3421010060	100 ul					H, L, I	Y / N / Unk
☐ SR27 3421010079	100 ul					H, L, I	Y / N / Unk
☐ SR28 3421010088	100 ul					H, L, I	Y / N / Unk
☐ SR29 3421010097	100 ul					H, L, I	Y / N / Unk
☐ SR30 3421010104	100 ul					H, L, I	Y / N / Unk
☐ SR31 3421010113	100 ul					H, L, I	Y / N / Unk
☐ SR32 3421010122	100 ul					H, L, I	Y / N / Unk
☐ SR33 3421010131	100 ul					H, L, I	Y / N / Unk
☐ SR34 3421010140	100 ul					H, L, I	Y / N / Unk
☐ SR35 3421010159	100 ul					H, L, I	Y / N / Unk
☐ SR36 3421010168	100 ul					H, L, I	Y / N / Unk
☐ SR37 3421010177	100 ul					H, L, I	Y / N / Unk
☐ SR38 3421010186	100 ul					H, L, I	Y / N / Unk
☐ SR39 3421010195	100 ul					H, L, I	Y / N / Unk
☐ SR40 3421010202	100 ul					H, L, I	Y / N / Unk

If all tubes were not created, give reason: