ADDENDUM 1: CHANGE IN PROTOCOL

Summary of Changes: Due to the poor yield on our eligibility criteria for early staged cancers, we have implemented the following changes. Most importantly, there will be a change in the processing of the blood that will allow for the recruitment of pancreatic cancer cases to the study following surgery. Previously, blood needed to be aliquoted into 100 microliter volumes (a total of 60 tubes), which was quite time consuming and differs from the how specimens are routinely stored at the participating sites. Since all of the centers routinely enroll pancreatic cancer cases into their own biospecimen bank and collect blood pre-operatively, it has been decided that we should identify the pancreatic cancer cases following surgical resection. A total of 4 ml of serum and 2 ml of plasma for patients found to have stage 1 or 2A cancers will be set aside for the study. The appropriate questionnaire will still need to be filled out for the PCCR to allow us to generate our control population. Blood from control cases will be processed the same as the PC cases (consistent within the institution). At the end of the study, 4 ml of serum and 2 ml of plasma will be sent to a central site (to be determined) to be aliquoted into the smaller volumes and recoded for the study. With this new recruitment strategy, all tumors should be stage 1 and 2A. Previously collected specimens will also be included in the validation set. A thousand dollars will still be given for each sample and paid when the blood has been sent to the central site and all associated paperwork completed.
1. Objectives

The primary objective of the EDRN Pancreatic Cancer Working Group Proposal is to create a reference set consisting of well-characterized serum/plasma specimens to use as a resource for the development of biomarkers for the early detection of pancreatic adenocarcinoma. The testing of biomarkers on the same sample set permits direct comparison among them; thereby, allowing the development of a biomarker panel that can be evaluated in a future validation study. Additionally, the establishment of an infrastructure with core data elements and standardized operating procedures for specimen collection, processing and storage, will provide the necessary preparatory platform for larger validation studies when the appropriate marker/panel for pancreatic adenocarcinoma has been identified.

2. Background

Pancreatic cancer is the 4th most common cause of cancer death in the United States despite being responsible for only 2% of all malignancies.\(^1\) It is anticipated in the United States that in the year 2007, 37,170 individuals will be diagnosed and 33,370 will die from this disease.\(^1\) The 5-year survival rate for adenocarcinoma of the pancreas is less than 5%, with most patients dying within the first 2 years.\(^1\) This dismal survival rate is largely due to our failure to diagnosis a cancer at an early stage when the option of curative resection is still possible. Even in the subset of patients with resectable disease, 5-year survival rates are suboptimal, ranging from 20-60%.\(^1,2\)

The best long-term survival rates are seen when the pancreatic cancer is less than 1 to 2 cm in size, and there is lack of involvement of lymph nodes.\(^3-5\) Important factors in determining prolonged survival following resection are lymph node status and tumor size. A study by Yeo et al.\(^3\) reported significantly better median survival (28 months vs. 13 months) and 5-year survival (36% vs. 14%) in patients with no lymph node metastases as compared to those having lymph node metastases. Tumor size is another factor that predicts better long-term survival. Arriyama et al.\(^4\) found in a study of 77 resected adenocarcinoma patients a postoperative 5-year cumulative survival rate of 100% for patients with tumors less than 1 cm. However, irrespective of tumor size, once a carcinoma was greater than 1.1 cm, there was no statistical difference in survival rates. Only 10% of patients in this highly selected population had a tumor < 1 cm in size. A study of patients with small pancreatic cancers that were resected and measured less than 2 cm in size from the Japanese National Pancreatic Cancer Registry illustrates the challenges of diagnosing early staged carcinomas.\(^5\) Of 822 patients with a tumor less than 2 cm, 799 underwent pancreatic resection and about half were UICC staged as Ia (n=197) or Ila (n=138). Corresponding 5-year survival rates of 49.4% and 41.4%, respectively, were seen. These 5-year survival rates were substantially better than for the other half of patients with more advanced UICC stages: IIb (17.1%), III (15.8%), and IV (9.4%). Although one would ideally want a study set consisting of tumors less than 1 cm in size with no lymph node metastases, this is not realistic due to the limited number of tumors detected at this size. Thus, a reasonable and practical initial goal for a reference set is the identification of UICC stage I and IIA pancreatic adenocarcinomas. The advantage of this approach is that it not only allows for detection of a tumor with a better opportunity for long-term survival (7 to 10-fold better than current rate less than 5%) but also permits specimen collection to be accomplished with a limited number of participating sites in a reasonable time course (12 to 18 months). Even with the proposed inclusion criterion that consists of tumors up to 4 cm in size with no lymph node metastases, a multi-center collaborative effort will be essential since no single center has enough of these early staged lesions (PanIN 3 lesions or stage I and IIA cancers) to evaluate potential early detection methods.

Commonly used imaging studies such as endoscopic ultrasound, abdominal CT or MRI are inadequate for the detection of pancreatic cancer at an early stage, since these modalities do not reliably detect pancreatic tumors <1-2 cm in size.\(^6\) The clinical role of molecular markers for the early detection of pancreatic cancer has been limited. CA 19-9, a sialylated Lewis\(^a\) antigen associated with circulating mucins, is the most widely used marker. Although CA 19-9 is most frequently increased in pancreatic adenocarcinoma, it may also be elevated in other malignancies, particularly those of the bile duct, stomach and colon, and benign conditions such as acute and chronic pancreatitis, biliary obstruction and hepatitis.\(^7,8\) Results from a
recent study by Kim et al. demonstrated the ineffectiveness of serum CA 19-9 as a screening test in an asymptomatic population since their positive predictive value was below 1%. The imperfect role (as evident by no significant improvement in the 5-year survival rate) of currently available imaging modalities and lack of available molecular markers of pancreatic cancer stress the importance of developing approaches that can detect pancreatic tumors when they are small and potentially curable by surgical resection.

2.1 Significance of Project

The GI section of the EDRN sponsored a workshop in Norfolk, VA in September of 2004 and in Denver, CO in January 2006 to discuss the current status of pancreatic biomarker development. Discussions at both of these workshops highlighted obstacles for investigators working in this field. A major conclusion was the need to develop reference sets consisting of earlier stages of pancreatic cancer when there was still an opportunity for curative resection and a cohort of well defined control cases. Furthermore, each case would be accompanied by meaningful clinical data (common data elements). It was recognized by the workshop participants that this effort would require a collaborative effort and needed to be done prospectively to ensure that the specimens were collected and processed in a standardized manner. The main goal of this proposal is the development of a narrow validation set that will identify biomarker(s) that eventually could be investigated, either alone or as part of a panel, in a larger validation set, which will need to be construed in the future. As a consequence of our study design, a second set of specimens will inadvertently be collected and processed from a subset of patients found during surgery to have more advanced disease (stage IIB and greater). Since the tumor stages of these specimens are too advanced for inclusion in the proposed validation reference set, it is planned that these specimens be used for a discovery set, which can serve as a tool for investigators to test promising biomarkers during the earliest phases of biomarker development. Both sets will be under the control of the EDRN and stored at the central NCI biorepository in Frederick, MD.

3.0 Eligibility Criteria (Appendix A)

3.1 Cases (Pancreatic Cancer)

Patients will be consented prior to surgery.
A. No prior history of any other malignancy except nonmelanoma skin cancers for ten years.
B. Must have undergone complete surgical resection of the tumor with curative intent including the presence of negative margins and a pathologic staging of I or IIA.
C. Must have histological verification of a pancreatic adenocarcinoma
D. Must not have received preoperative chemoradiation (neoadjuvant) therapy due to our inability to adequately stage these patients.
E. Able to give informed consent.
F. Blood sample must be obtained within the past 2 weeks prior to surgery/anesthesia according to procedure described in Appendix D.

Post-Enrollment Criteria: Inclusion of the patient in the proposed validation set is dependent on the results of the surgery. The patient must have undergone complete surgical resection of the tumor with curative intent including the presence of negative margins and a pathologic staging of I or IIA. Samples from any patients not meeting these criteria will be used in a discovery set.

3.2 Controls

Three different control groups are proposed for this reference set: chronic pancreatitis patients, acute benign biliary obstruction patients and healthy controls. The cases and controls are required to have a similar age distribution. Although we will attempt to keep the same number of cases and controls within each site (1 to 3 ratio), age distribution does not have to be similar for cases and controls within each site. An algorithm developed by Feng and colleagues will be used to recruit cases and controls across multi-centers to ensure that the cases and controls are frequency matched by age. Eligibility criteria for each of
the controls groups are described below. Control specimens will not be collected until 10 post-operative PC cases have been successfully enrolled in the study.

Subjects with chronic pancreatitis:
A. All subjects must have at least two of the radiological criteria listed below, unless a subject has a history of pancreatic exocrine insufficiency in which case only one radiological criterion is required.
   1) Abdominal ultrasound that is consistent with chronic pancreatitis by standard radiological criteria (i.e. echogenic foci in the parenchyma, large or small cavities, calcifications, dilated pancreatic duct).
   2) Abdominal CT scan consistent with chronic pancreatitis by standard radiological criteria (i.e., calcifications, dilated pancreatic duct, irregular contour of the gland, cystic lesions).
   3) ERCP exam consistent with chronic pancreatitis by standard radiological criteria (dilated tortuous main pancreatic duct with irregular secondary branches, intraductal calculi).
   4) Endoscopic ultrasound consistent with chronic pancreatitis by standard radiological criteria (echogenic foci, focal regions of decreased echogenicity, pancreatic ductal changes).
   5) Pancreatic calcifications identified on plain film of the abdomen.
B. Must have an imaging study of the pancreas within 3 months of study enrollment which does not suggest a pancreatic mass.
C. Stable clinical history over the past year with no suspicion for cancer (weight loss, jaundice, or change in abdominal symptoms).
D. Age matched to qualified pancreatic cancer cases.
E. No prior history of any other malignancy except non melanoma skin cancers for the past ten years.
F. No family history of pancreatic cancer.
G. Able to give informed consent.

Subjects with acute benign biliary obstruction
A. Must meet all of the following clinical criteria:
   1) Elevation of serum bilirubin level greater than 2.0 mg/dL
   2) Dilated extrahepatic biliary systems demonstrated on US, MRI or CT scan
   3) Blood sample obtained within 72 hours of admission and prior to any corrective intervention.
B. Biliary obstruction must be of benign etiology such as CBD stone or benign biliary stricture.
C. Patients with primary sclerosing cholangitis (PSC) will be excluded.
D. Must have complete imaging study performed of the pancreas that does not suggest a pancreatic cancer (discrete mass lesion)
E. Age matched to qualified pancreatic cancer cases.
F. No prior history of any other malignancy except non melanoma skin cancers for the past ten years.
G. No family history of pancreatic cancer.
H. Must be able to give informed consent.

Healthy controls:
A. Age, race and sex matched to qualified pancreatic cancer cases.
   1) Age will be matched by 5 year intervals to a pancreatic case (i.e. 40-44; 45-49; 50-54; 55-59; etc.).
B. No family history of pancreatic cancer.
C. No personal history of acute pancreatitis or biliary obstruction as defined above.
D. No concurrent abdominal pain.
E. No concurrent unexplained weight loss.
F. No prior history of any other malignancy except non melanoma skin cancers for the past ten years.
G. Must be able to give informed consent.
4.0 Sample Size Justification

Sample size calculations were conducted using methods suggested in Pepe (2003, pages 220-224). If we set our criterion for access to the reference set, i.e., the minimally acceptable marker performance, as TP=0.6 and FP=0.4, and we hope to demonstrate that statistically the marker’s performance is at least TP=0.8, FP=0.2, with 90% power and alpha level = 0.10, we will need 60 cases and 60 of each of the controls. We selected the lower boundary to be relatively low compared with other cancer sites as currently, there are no markers available for pancreatic cancer screening that demonstrate a satisfactory performance. We selected the upper boundary based on the performance of CA-19-9 as the only available diagnostic marker for PC (recognizing that these values for CA19-9 are based on detecting all cases of pancreatic cancer, the majority of which are metastatic at presentation). We set the rejection level at 0.10, since this is a slightly more liberal rejection criterion and would lower our chance of missing potential good markers at this early pre-validation stage. Therefore, we elect to build the reference set of specimens with 60 cases, and 60 healthy controls, 60 with acute biliary obstruction, and 60 with chronic pancreatitis. Case and control samples will be matched on age, race, study site and gender.

5.0 Overview of patient recruitment

5.1 Cases
Pancreatic cancer patients will be identified prior to surgical resection. All blood will be obtained after the patient has met the eligibility criteria, signed IRB-approved consent forms and prior to surgery. Questionnaires will be filled out with the assistance of personnel who will undergo training to ensure a standardized approach between the participating centers. Staging will be determined at the time of surgery. Pathology reports (minus patient identifiers) and questionnaires will be reviewed at a central site by the PCCR coordinator who will verify that the information was accurately entered into the database (double data entry). Specimens will be collected from 60 Stage 1 and 2A pancreatic cancer patients.

5.2 Controls
Subjects will be identified at the sites according to the eligibility criteria listed in section 3.2. All blood will be obtained after the subject has met the eligibility criteria and signed IRB-approved consent forms. Questionnaires will be filled out with the assistance of personnel who will undergo training to ensure a standardized approach between the participating centers. Questionnaires will be reviewed at a central site by the PCCR coordinator who will verify that the information was accurately entered into the database.

6.0 Data collection and Storage

This project will utilize a NIH-funded web-based infrastructure, the Pancreatic Cancer Collaborative Registry (PCCR), which is currently being implemented by the University of Nebraska Medical Center Eppley Cancer Center to manage clinical data for both sporadic and hereditary pancreatic cancer patients and their families. Every participating site with the exception of Memorial Sloan Kettering is a member of the PCCR. As a member the participants have agreed to collect common data elements (CDEs) on their pancreatic cancer patients and abide by the PCCR steering committee approved set of bylaws. Furthermore, participants already have IRB approval to share de-identified data in the PCCR for approved projects. The PCCR CDEs are compatible with EDRN CDEs. Since samples will be collected prospectively, the minimum core data elements required for pancreatic cancer and control cases will consist of the EDRN core elements as shown in the Data Collection Form (Appendix B). However, for pancreatic cancer cases, sites will be encouraged to collect as many of the additional elements included in the PCCR database. The PCCR is actively expanding at the time of this submission and presently has 7 committed sites. These sites are University of Nebraska Medical Center, Evanston Northwestern Healthcare, Creighton University, University of Pittsburgh, University of Alabama Birmingham, University of Michigan and the University of Genoa, Italy.

Sites Confirmed to Participate in this proposal: Evanston Northwestern, University of Alabama Birmingham, University of Nebraska, University of Michigan, Memorial Sloan Kettering and University of Pittsburgh
Potential Sites to participate in this proposal: MD Anderson and Johns Hopkins (if these sites participate they will join the PCCR).
Sites Not Participating in this proposal: Creighton University and University of Genoa, Italy.

All recruiting sites will collect the CDEs on hardcopy, enter the CDEs into the PCCR database and send a copy of the forms to the PCCR clinical coordinator, Marsha Ketcham for quality assurance review and double-entry of the data into the PCCR. The data will be transferred to the Data Management and Coordinating Center (DMCC) on a to-be-determined basis and the DMCC will develop a control recruitment algorithm. A description of the PCCR is in Appendix C.

7.0 Sample Collection and Processing Requirements
All blood samples will be collected according to the newly created EDRN standard operating procedure (See Appendix D). All samples will be frozen at -70 degrees or colder within 4 hours of time of collection. A minimum of 30 mls of blood will be drawn including two 10 ml red top glass tube and one 10 ml of EDTA plastic tube. This should yield 8 ml of serum and 4 ml of plasma. The samples will be stored at whatever volume desired by the recruiting site for the pancreatic cancer cases and control cases (should be the same). A total of 4 ml of serum and 2 ml of plasma from eligible pancreatic cancer cases and recruited controls will be sent to a central site to be aliquoted into:

(40) 100 μl aliquots of serum
(20) 100 μl aliquots of plasma

Any remaining plasma/serum/buffy coat will be kept at the center which recruited the patient. Ideally, blood will be obtained fasting; however if necessary, non-fasting samples will be used. Fasting status of a patient must be tracked.

Sample Storage Conditions
Specimens will initially be stored at the collecting center at -70°C or colder. All specimens will be bar coded under the direction of the DMCC in a manner that will allow for the identification of the site and the associated common data elements. The DMCC will develop a Specimen Tracking System within the Validation Study Information Management System (VSIMS) for sites to enter and associate all specimens to the PCCR participant ID. Only specimens being transferred to NCI-Frederick will be entered into VSIMS. Each recruiting site will receive a set of 2-D barcode labels from the DMCC to affix to the Vacutainer Tubes and Aliquots. Each site will need to purchase a 2-D barcode scanner if one is not currently available. Specimens will be transferred to the central facility in a manner which will prevent thawing using approved transporter techniques.

8.0 Process for utilization of the samples
Determination for utilization of the specimens will be overseen by the GI section of the EDRN following previously established EDRN guidelines.

9.0 Ethical and Regulatory Considerations

9.1 Protection of human subjects
All patients will undergo informed consent to participate in this project. Each site will have institutional IRB approval that will permit the sharing of de-identified data and blood specimens. It will not be possible to identify any participant in this study or link them with any results from future biomarker analysis.

9.2 Data safety monitoring
Data safety monitoring to ensure compliance with patient anonymity will be performed by the principal investigator with the assistance of the PCCR project coordinator. Furthermore, monitoring will also be performed by the DMCC and each participating center’s IRB.

10.0 Budget Overview
It is proposed that a center will be reimbursed $1,000 (direct and indirect costs) for every specimen forwarded to the repository at Frederick, MD. (250 subjects- it is anticipated that more cases will be required since many pancreatic cancer patients will have more advanced disease despite our use of strict eligibility criteria).
10% effort for PI to oversee the complete project and oversee recruitment and specimen selection at his site. Each site will be responsible for determining the individual(s) who will receive this support. Responsibility of the co-PIs includes overseeing the successful implementation of the grant at their institution including patient recruitment, specimen handling and data collection. $1,000 will be given to each site for general supplies including bar coding equipment.

30% effort for PCCR clinical coordinator to oversee data collection including accuracy of data transfer from questionnaire.

10% effort for co-PI, Simon Sherman to oversee PCCR efforts in implementing the project.

25% effort for a PCCR computer programmer to assist in data management at each site as well as the transfer of data to the DMCC.

12.0 References

Appendix D:
Revised 06-13-08 REFERENCE SET – BLOOD

Will be adapted to conform to EDRN SOP for collection of blood specimens
NCI/EDRN/SPORE Pancreatic Cancer Proteomics Committee Specimen collection recommendations
Adapted from NCI/FDA Proteomics Program Protocol

Standard Operating Procedure
Collection of Serum and Plasma samples for Proteomic Analysis

I. 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.

II. Materials
1. Requisition
2. Gloves
3. Sharps Container
4. Vacutainer needles, 18g (reduces hemolysis) and vacu-fan hub or Butterfly needle, attached tubing and Luer adapter
5. Tourniquet
6. Antiseptic wipes
7. Serum: Two 10 ml (draw) red top glass tubes, no additive, no clot activator with silicone coated interior, (BD366430)
8. Plasma-EDTA: One 10 ml (draw) EDTA plastic tube (BD366643)
9. Aliquot containers for all blood specimens -.5 ml Polypropylene Micro Tubes, screw top, conical skirted (Sarstedt 72.730)
10. Bandages
11. Centrifuge (refrigerated or non-refrigerated)

III. Specimens
The specimen of choice for proteomic analysis at the NCI/FDA Proteomics Program is serum obtained from whole blood collected in red top vacutainer tubes with no additives or clot activators. The blood specimen should be allowed to clot for 40 – 50 minutes with tube upright. The serum should be transferred to a transfer/storage tube and frozen within 4 hours of collection (optimal). For difficult to obtain samples up to 12 hours may be permitted before freezing. Samples are then frozen at -70°C or colder until testing is performed. The serum should be free of hemolysis and clots by visual inspection. Freezing and thawing cycles should be kept to a minimum. (Plasma samples can also be analyzed, however, every sample in the set must be plasma and the same anticoagulant used for all samples in a set (e.g., all EDTA). The following serum and plasma procedures are based upon collecting at least 30 ml of whole blood from a patient.

IV. Procedure
1. Identification of the patient is crucial. The person obtaining the blood specimen must ensure that the blood specimen being drawn is from the individual designated on the request form.
2. Information regarding fasting status should be recorded at time of blood draw. Ideally, whenever possible patient should be fasting.
3. Assemble the supplies to be used in obtaining the specimen. Label the tubes.
4. Put on disposable gloves.
5. 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.
6. 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.
7. Clean the forearm of the patient with antiseptic wipe in a circular motion beginning at the insertion site. Allow the antiseptic to dry.
8. 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.
9. 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.
10. Release the tourniquet once blood flow is established.
11. 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.
12. Lightly place a sterile gauze pad over the venipuncture site. Gently remove the needle.
13. Apply pressure to the site with sterile gauze. Apply bandage. Instruct the patient to leave the bandage on for at least 15 minutes.
14. Dispose of the needle in a sharps container.
15. Remove gloves and wash hands.

Serum:
1. Carefully label tubes and aliquot tubes. Record time of draw and time of freezing.
2. Approximately 20 ml of blood is collected in 2 sterile 10 ml (draw) vacutainers (recommended BD Bioscience 366431, red top no additive vacutainer). This should yield 8 ml of serum.
3. Mix specimen thoroughly. Leave tubes upright on the bench at room temperature for ~ 45 minutes to allow clot to form.
4. Centrifuge at 1200xG for 10 minutes.
5. The samples will be stored at whatever volume desired by the recruiting site for the pancreatic cancer cases and control cases (should be the same). These can be stored temporarily on wet ice, but should be stored as soon as possible at -70°C or colder. Specimens should be frozen so that there is no more than 4 hours between drawing the specimen and freezing. A total of 4 ml of serum from eligible pancreatic cancer cases and recruited controls will be sent to a central site to be aliquoted into: (40) 100 μl aliquots of serum. Any remaining plasma/serum/buffy coat will be kept at the center which recruited the patient. Ideally, blood will be obtained fasting; however if necessary, non-fasting samples will be used. Fasting status of a patient must be tracked.
6. Dispose of all tubes and materials used to transfer patient samples in biohazardous waste.

Plasma and Buffy Coat:
1. Under the direction of a qualified and licensed physician, trained nurses, phlebotomists or others will collect blood from each donor into a vacutainer that has EDTA bound to its surface.
2. Carefully label tube and aliquot tubes including the types of anticoagulant for plasma. Keep a record from time of draw to time of freezing the aliquot.
3. From each consenting donor, 10 ml of EDTA plasma will be obtained. Mix specimens thoroughly after drawing.
4. The specimens are centrifuged immediately after blood draw in a centrifuge at 1200XG for 10 minutes. The resultant plasma (assume 40% yield) is transferred into secondary tubes.
5. The secondary tubes are then centrifuged at 1500 g for 5 minutes to remove all potentially remaining cells.
6. After mixing thoroughly again, the samples will be stored at whatever volume desired by the recruiting site for the pancreatic cancer cases and control cases (should be the same). These can be stored temporarily on wet ice, but should be stored as soon as possible at -70°C or colder. Specimens should be frozen so that there is no more than 4 hours between drawing the specimen and freezing. A total of 2 ml of plasma from eligible pancreatic cancer cases and recruited controls will be sent to a central site to be aliquoted into: (20) 100 μl aliquots of
plasma. Any remaining plasma/serum/buffy coat will be kept at the center which recruited the patient. Ideally, blood will be obtained fasting; however if necessary, non-fasting samples will be used. Fasting status of a patient must be tracked.

V. Procedural Notes:
1. Do not draw from an IV, mastectomy or shunt arm.
2. Samples may also be drawn into a syringe and then dispensed into a vacutainer tube (before clotting) for processing.
3. ORDER OF DRAW: Blood collection tubes must be drawn in a specific order to avoid cross-contamination of additives between tubes. The recommended order of draw is:
   First – non-additive tube (red stopper or SST)
   Second – EDTA (lavender stopper)
   If this is not possible because of drawing clinical samples first, please note the order of clinical sample drawing in your record and still draw the research EDTA sample after the research red top.

VI. Freeze thaw cycles should be minimized; however, up to 3 freeze thaws are acceptable. Each freeze thaw should be recorded in the sample history along with time the blood draw is complete and time of freezing. To minimize freeze thaws, a total of 4 ml of serum and 2 ml of plasma from eligible pancreatic cancer cases and recruited controls will be sent to a central site to be aliquoted into: (40) 100 μl aliquots of serum (20) 100 μl aliquots of plasma. Any remaining plasma/serum/buffy coat will be kept at the center which recruited the patient. Our assumptions are that each tube draw as described by Becton-Dickinson for serum or plasma will be 40% of blood samples. Also, an assumption is that serum will be in greatest demand for most studies.

NOTE: In the four hour period between collection and freezing, there will be unfortunately numerous uncontrolled variables. Thus there is no need for refrigerated centrifuges (10 minute spins) which are expensive and bulky.

The address of the storage facility is as follows:

Please inform the facility before shipping and plan to ship specimens only on Monday through Wednesday.