

# Investigations into the Effects of Blood Specimen Handling Procedures on Protein Integrity

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# Overview of project

Recruit 250 subjects of breast and prostate cancer at varying stages; single draw

- 1) Vary collection as well as plasma and serum preparation procedures in a controlled and randomized manner
  - a. Blood collection tube types
  - b. Use of protease inhibitors
  - c. Time and temperatures pre- and post-centrifugation
  - d. Number of freeze-thaw cycles
  - e. Time in -80°C freezer
- 2) Examine protein abundances and modifications (e.g. oxidation, proteolysis)
- 3) Use two technology platforms:
  - a. Label-free liquid chromatography-mass spectrometry (LC-MS) differential expression
  - b. Multiplexed Immunoassay (IA, using Luminex technology)
- 5) Correlate with other laboratory measures and patient information
- 6) Establish database of protein concentrations and modifications as metrics of sample integrity
- 7) From this database, establish a representative panel for efficient measurement and widespread use by multiplexed multiple-reaction monitoring (MRM) LC-MS
- 8) Evaluate numerous existing collections using this panel



# Research Team in addition to Caprion

## **Patient Recruitment and Phlebotomy**

### **Palo Alto Medical Foundation (PAMF)**

Arthur Bobrove, M.D., Director of Research

## **Multiplexed Immunoassay (IA) Development**

### **Millipore Corp. (using Luminex xMAP Technology)**

David Hayes, Ph.D.

Director R&D, Bioscience Division

Jehangir Mistry, Ph.D.

Director, R&D, Bioscience Division

Linda Meeh, Ph.D.

Director, Protein Research Assay Group

Including resources from the former Linco and Upstate sites



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# Research Team at Caprion

Sample manipulations

LC-MS measurements

IA measurements

Statistics

Targeted MRM Panel Development

Testing of Existing Collections

Reporting

Key individuals:

Hua Lin, Ph.D., Associate Director

Ted Jones, Ph.D., Senior Biostatistician



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# Plasma and Serum collection variables

- 1) Number of subjects 250; half breast cancer subjects, half prostate cancer subjects
- 2) Single draw. N = 25 for individual variables (tube types, times, temperatures)
- 3) Blood collection tube type (5 varieties)
- 4) Use/non-use of protease inhibitors
- 5)  $T_b$ = Blood time from draw to centrifugation (0.5 to 24 hours)
- 6)  $T_p$ = Plasma time from centrifugation to pipetting (& freezing) (0.5 to 24 hours)
- 7)  $T_f$ = Time in freezer (to 3 years, plus archived samples)
- 8)  $N_c$ = Number of freeze-thaw cycles (1 to 5)



# LC-MS Profiling of Modifications: basics of analysis

Track, identify and differentially quantify ~ 15,000 molecular ions per sample

One-dimensional LC-MS analysis with 14 most abundant proteins depleted

Post-translational modifications included

False discovery rate for identification < 1% using a decoy database



# LC-MS Profiling of Modifications: primarily proteolysis and oxidation

Antithrombin-III (ATIII)

FATTFYQH

FATTFYQHLAD

FATTFYQHLADSK

NDNDNIFLSPL

NDNDNIFLSPLSISTAF

NDNDNIFLSPLSISTAFAMTK

TSDQIHFF

TSDQIHFFF

TSDQIHFFFAK

Gelsolin (Actin-depolymerizing factor)

VPFDAATLH

VPFDAATLHT

VPFDAATLHTSTA

VPFDAATLHTSTAM

VPFDAATLHTSTAMAAQHGMDDDGTTGQK



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# Multiplexed Immunoassay Panels

Migration Inhibitory Factor (MIF)

Leptin

Prolactin

Osteopontin

IL-6

Cancer Antigen CA-125

Prostate Specific Antigen (PSA)

Carcino-embryonic antigen (CEA)

Alpha fetoprotein (AFP)

Oncofetal Antigen CA-19-9

Cancer Antigen CA-15-3 (Mucin 1)

Coagulation factor XIII

Antithrombin-III (ATIII)

Complement factor H-related protein 1 (FHR-1)

Extracellular matrix protein 1 (Secretory component p85)

Ficolin-3 (Collagen/fibrinogen domain-containing protein 3)

Gelsolin (Actin-depolymerizing factor / ADF)

Vitamin D binding protein

Vitronectin

Lumican

Actin-beta cytoplasmic 1



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# Clinical Collection Informatics

- Computer systems can help assist with and ensure the details of clinical collections
- Tailored for this collection of plasma and serum
- Barcoding of all tubes, and aliquots, as well as down-stream sample processing
- Handling of samples and timing of manipulations
- Integrity of sample tracking
- Use of collection kit which has all required components, with all tubes barcoded



# LIMS Tree View

The screenshot shows a LIMS interface with a tree view on the left and a table on the right. The tree view is expanded to show a study (P3874PAM001) with a subject (P3874PAM001\_02PL) and multiple draw/time-points (P3874PAM001\_02PL01 to P3874PAM001\_02PL09). The table on the right lists the tests and their external reference numbers.

**Study** (points to P3874PAM001)

**Subject** (points to P3874PAM001\_02PL)

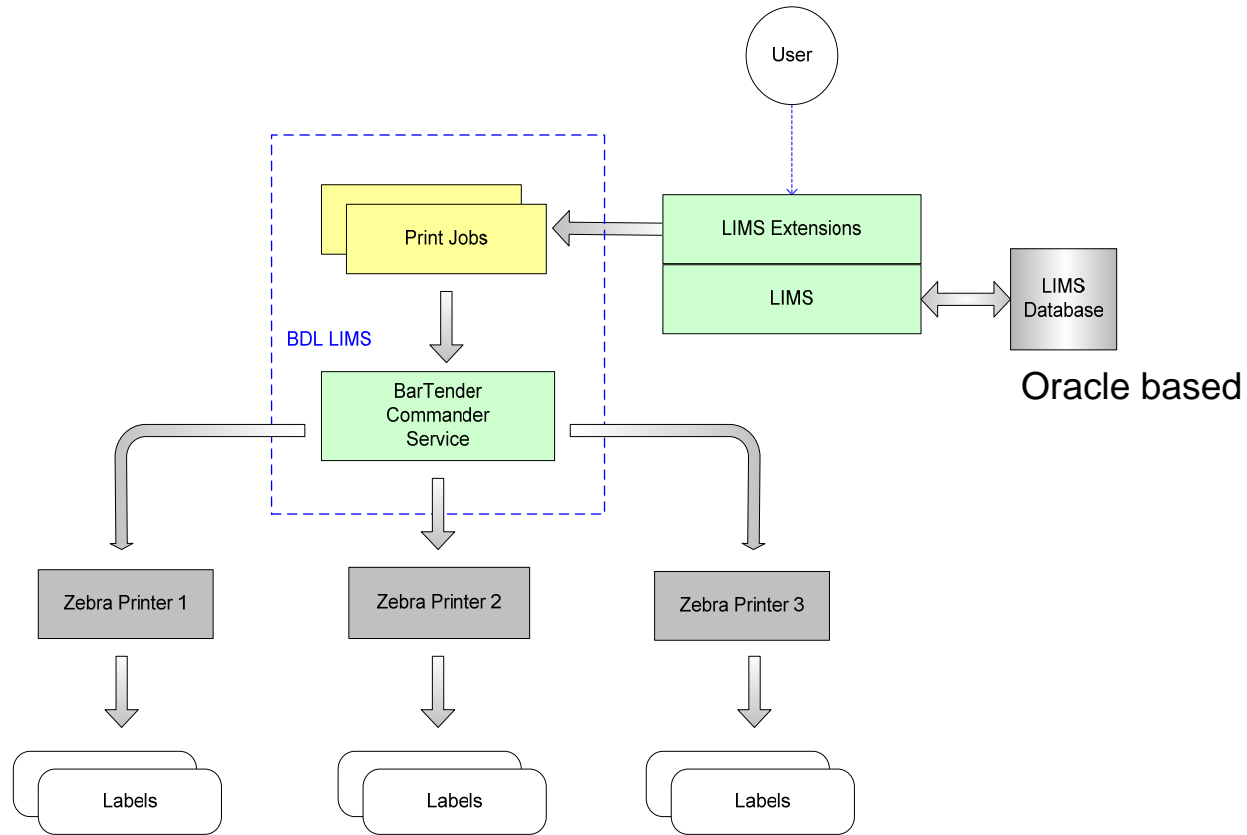
**Draw/time-point** (points to P3874PAM001\_02PL01)

**Frozen aliquot** (points to P3874PAM001\_02PL01 to P3874PAM001\_02PL09)

Name	Project	External Re..
PLasmaTest		
P3874PAM001_02PL01		574137
P3874PAM001_02PL02		574138
P3874PAM001_02PL03		574139
P3874PAM001_02PL04		574140
P3874PAM001_02PL05		574141
P3874PAM001_02PL06		574142
P3874PAM001_02PL07		574143
P3874PAM001_02PL08		574144
P3874PAM001_02PL09		574145



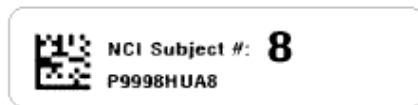
# LIMS Barcode Label System v2.0



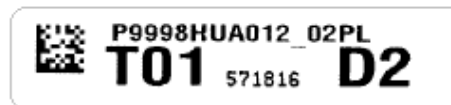
## Two-dimensional barcodes

Acknowledgment to Mark Cosentino, SAIC

Subject Label



Draw Label  
(5 per subject)



Frozen Aliquot Label  
(5-9 per draw)



# Tube Labeling and Kit Preparation



Not shown: Subject interview form, phlebotomy accessories,  
subject barcode for log book

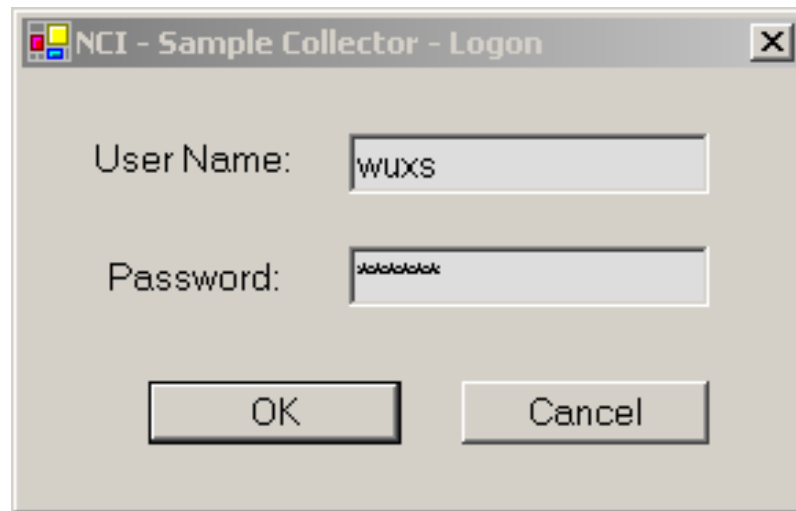
# Sample Collector Program

- Tablet PC program used to assist in the clinical collection
- Recording of subject information and blood collection information
- Has timing requirements and prompts (beeps and flashing lights!)
- Automatic frequent file backup
- Subject and collection information for subsequent statistics
- Written in C#/.net language



Caprion Proteomics  
SAIC/NCI Biospecimen Integrity Project –  
SCREEN SHOTS for SAMPLE COLLECTOR PROGRAM

Sample Collector – Login



NCI - Sample Collector - Logon

User Name:

Password:

# NCI Biospecimen Integrity Project – Sample Collector – Subject Page/Tab

NCI Biospecimen Integrity Project - Sample Collector

**Subject Information** NCI Project: R&D on Human Biospecimen Integrity

Scan Subject (1st Draw) Barcode:  PAMF Subject Code #:  Date/Time:

Subject ID:  Collection Tube Type (5 varieties):

Signed Informed Consent Received?  Yes  No

Year of Birth:  Gender:  Male  Female

Height:  feet  inches Ethnic Category:

Weight:  pounds Racial Category:

Last Time Had Meal:  Any Acute Sickness Today:  Yes  No

Approx Date of Initial Cancer Diagnosis:  Current Stage of Disease:

Current Cancer Treatments (last 3 months):

Other Current or Recent Medications (last 30 days):

Other Active Health Problems:

# Sample Page – Tb 1 Time Due, Timer and Button Turns Yellow, Flashes and Emits Repeating Warning Noise

NCI Biospecimen Integrity Project - Sample Collector

Subject: **Sample Tracking**    **STUDY 1 - Tb**    Sample Collected, Patient Departed    Current Time: **4:48:01 PM**

Step 1: Arrange 5 draw tubes following this map, and scan draw barcodes on each tube:

Draw #:	D2	D4	D3	D5	D1
Time Point:	T1	T2	T3	T4	T5
Draw Barcode:	574324 P3874PAM005_02	574326 P3874PAM005_04	574325 P3874PAM005_03	574327 P3874PAM005_05	574323 P3874PAM005_01

Step 2: Enter minutes since sample was drawn:  And click:     Tb started at: **4:17:55 PM**

Step 3: Enter Blood Level (cm):

<input type="text" value="8.5"/>	<input type="text" value="8.2"/>	<input type="text" value="8.3"/>	<input type="text" value="8.4"/>	<input type="text" value="7.5"/>
----------------------------------	----------------------------------	----------------------------------	----------------------------------	----------------------------------

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**Tb Timing - Blood Draw to Centrifugation**

	Tb 1	Tb 2	Tb 3	Tb 4	Tb 5
Required Blood Time:	<input type="text" value="30 Minutes"/>	<input type="text" value="1 Hour"/>	<input type="text" value="4 Hours"/>	<input type="text" value="24 Hours"/>	<input type="text" value="24 Hours (4°C)"/>
Count Down:	<b>00:06 OVER DUE</b>	<b>29:53</b>	<b>3:29:53</b>	<b>23:29:53</b>	<b>23:29:53</b>
When a timer is due (turns yellow), click button to proceed to centrifugation:	<input type="button" value="Start Centrif"/>	<input type="button" value="Start Centrif"/>	<input type="button" value="Start Centrif"/>	<input type="button" value="Start Centrif"/>	<input type="button" value="Start Centrif"/>

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**Tp Timing Plasma Centrifugation to Pipetting**

	Tp 1	Tp 2	Tp 3	Tp 4	Tp 5
Required Plasma Time:	<input type="text" value="30 Minutes"/>	<input type="text" value="30 Minutes"/>	<input type="text" value="30 Minutes"/>	<input type="text" value="30 Minutes"/>	<input type="text" value="30 Minutes"/>
Count Down:	<input type="text" value=""/>	<input type="text" value=""/>	<input type="text" value=""/>	<input type="text" value=""/>	<input type="text" value=""/>
When a timer is due (turns red), click button to proceed to Pipetting:	<input type="button" value="Start Pipetting"/>	<input type="button" value="Start Pipetting"/>	<input type="button" value="Start Pipetting"/>	<input type="button" value="Start Pipetting"/>	<input type="button" value="Start Pipetting"/>
	<input type="checkbox"/> Centrif Done	<input type="checkbox"/> Centrif Done	<input type="checkbox"/> Centrif Done	<input type="checkbox"/> Centrif Done	<input type="checkbox"/> Centrif Done
	<input type="checkbox"/> Pipetting Done	<input type="checkbox"/> Pipetting Done	<input type="checkbox"/> Pipetting Done	<input type="checkbox"/> Pipetting Done	<input type="checkbox"/> Pipetting Done
	<input type="checkbox"/> Sample in Freezer	<input type="checkbox"/> Sample in Freezer	<input type="checkbox"/> Sample in Freezer	<input type="checkbox"/> Sample in Freezer	<input type="checkbox"/> Sample in Freezer



# Fills in "Aliquots" Page

NCI Biospecimen Integrity Project - Sample Collector

Subject | Sample | Frozen Aliquot | Notes

### T01 Aliquots

Aliquot#	Barcode	Volumn
1	576312	250 µl
2	576313	250 µl
3	576314	250 µl
4	576315	1.5 ml
9	576320	1.2 ml

Complete

Update...

Photo Taken

### T02 Aliquots

Aliquot#	Barcode	Volumn
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Incomplete

Update...

Photo Taken

### T03 Aliquots

Aliquot#	Barcode	Volumn
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Incomplete

Update...

Photo Taken

### T04 Aliquots

Aliquot#	Barcode	Volumn
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Incomplete

Update...

Photo Taken

### T05 Aliquots

Aliquot#	Barcode	Volumn
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Incomplete

Update...

Photo Taken

# “Notes” Page

NCI Biospecimen Integrity Project - Sample Collector

Subject

Sample

Frozen Aliquot

Notes

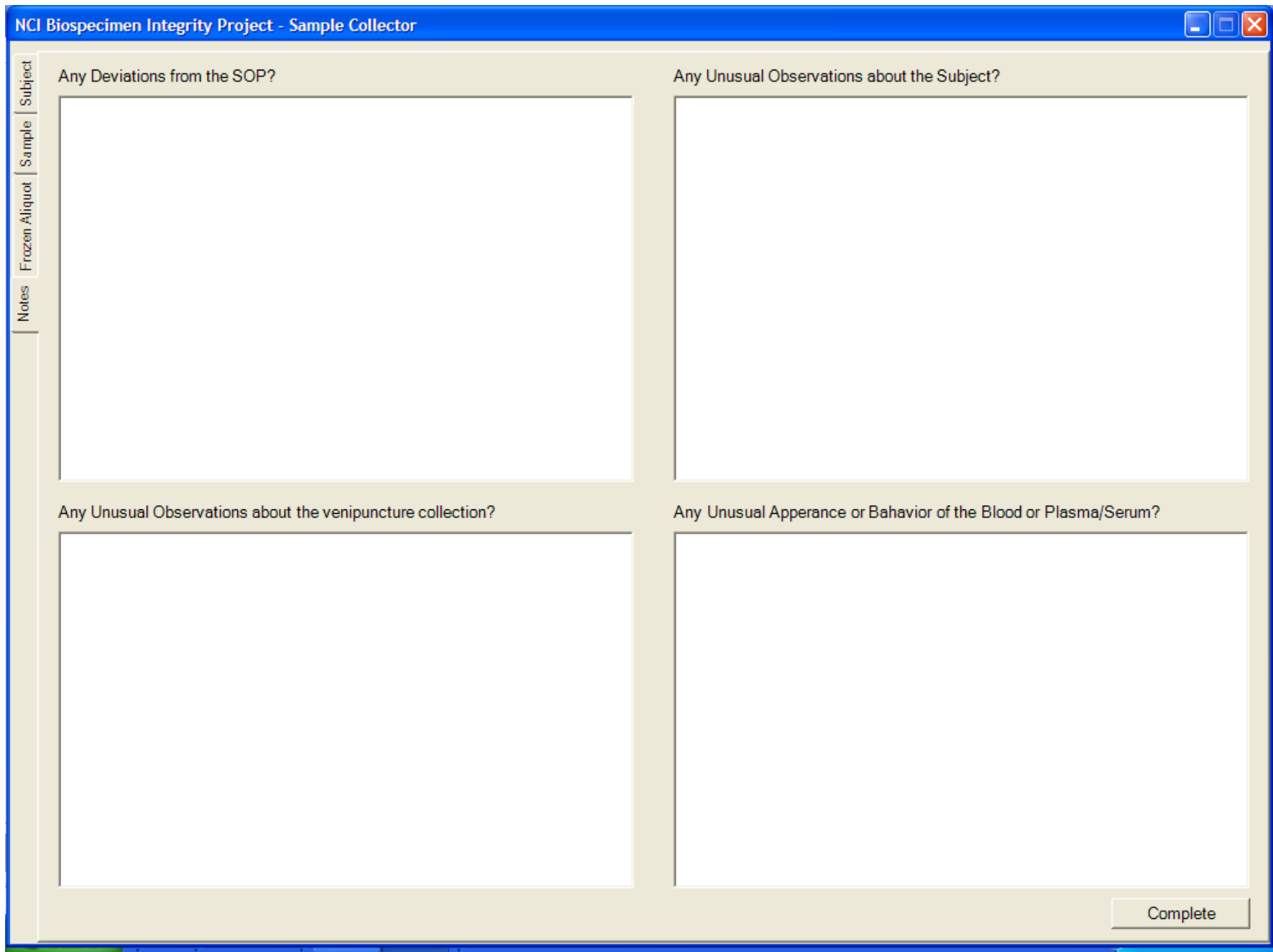
Any Deviations from the SOP?

Any Unusual Observations about the Subject?

Any Unusual Observations about the venipuncture collection?

Any Unusual Appearance or Behavior of the Blood or Plasma/Serum?

Complete



# Summary

Quantitatively evaluate major pre-analytical variables for plasma/serum collection and how they affect detailed molecular information

Perform LC-MS and Immunoassay protein measurements

Establish a database of extensive molecular measures, and then

Establish a standard panel of markers with which to grade blood protein integrity using multiplexed targeted (MRM) LC-MS assay

Apply standard panel to evaluate existing collections

Enable widespread use of this standard panel



END



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