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

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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) Tb= Blood time from draw to centrifugation (0.5 to 24 hours)
6) Tp= Plasma time from centrifugation to pipetting (& freezing) (0.5 to 24 hours)
7) Tf= Time in freezer (to 3 years, plus archived samples)
8) Nc= Number of freeze-thaw cycles (1 to 5)

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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

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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
VPFDAATLHTSTAMAAQHGMDDDGTGQK

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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 cystoplasmic 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

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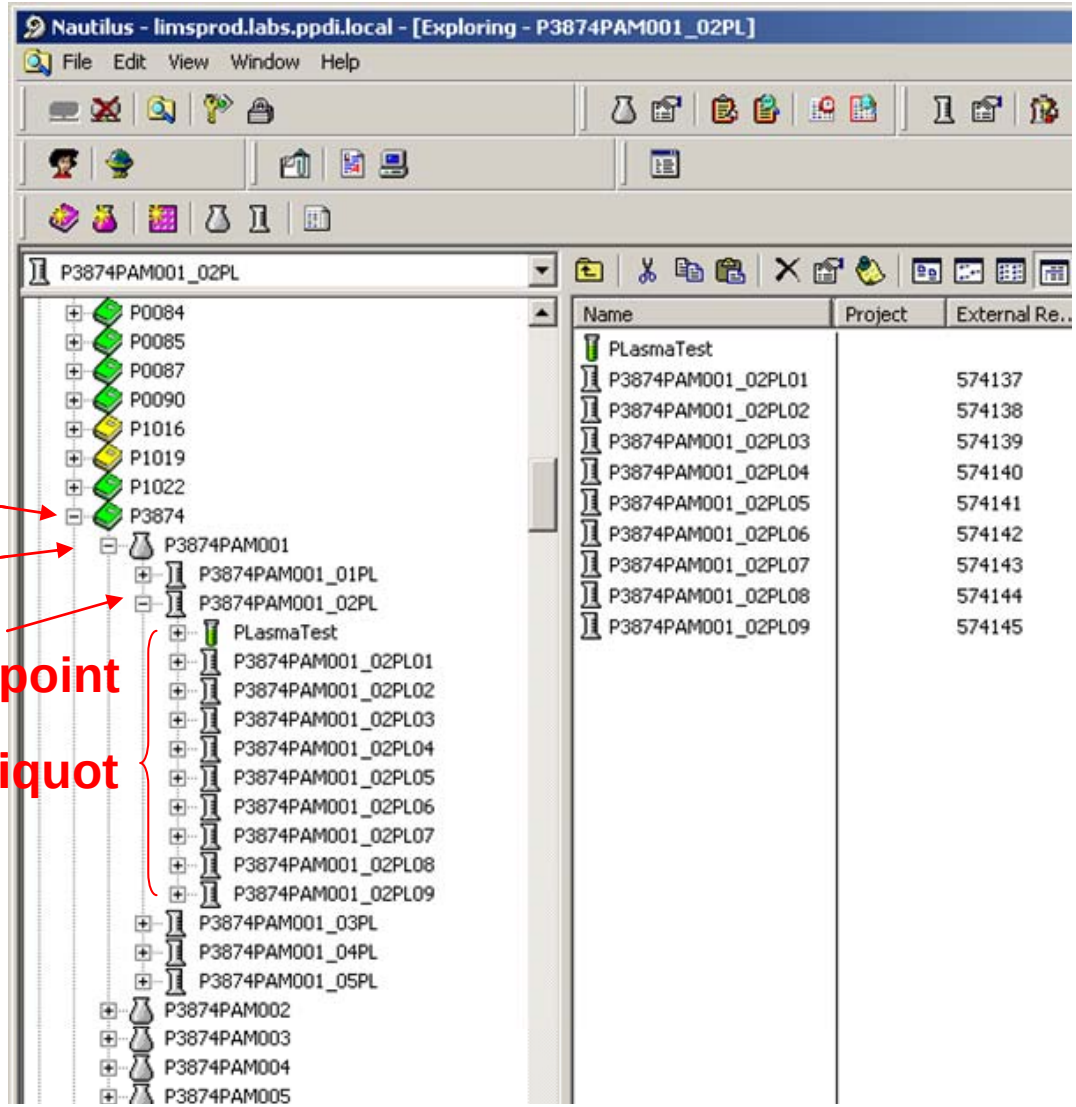
LIMS Tree View



Study

Subject

Draw/time-point

Frozen aliquot

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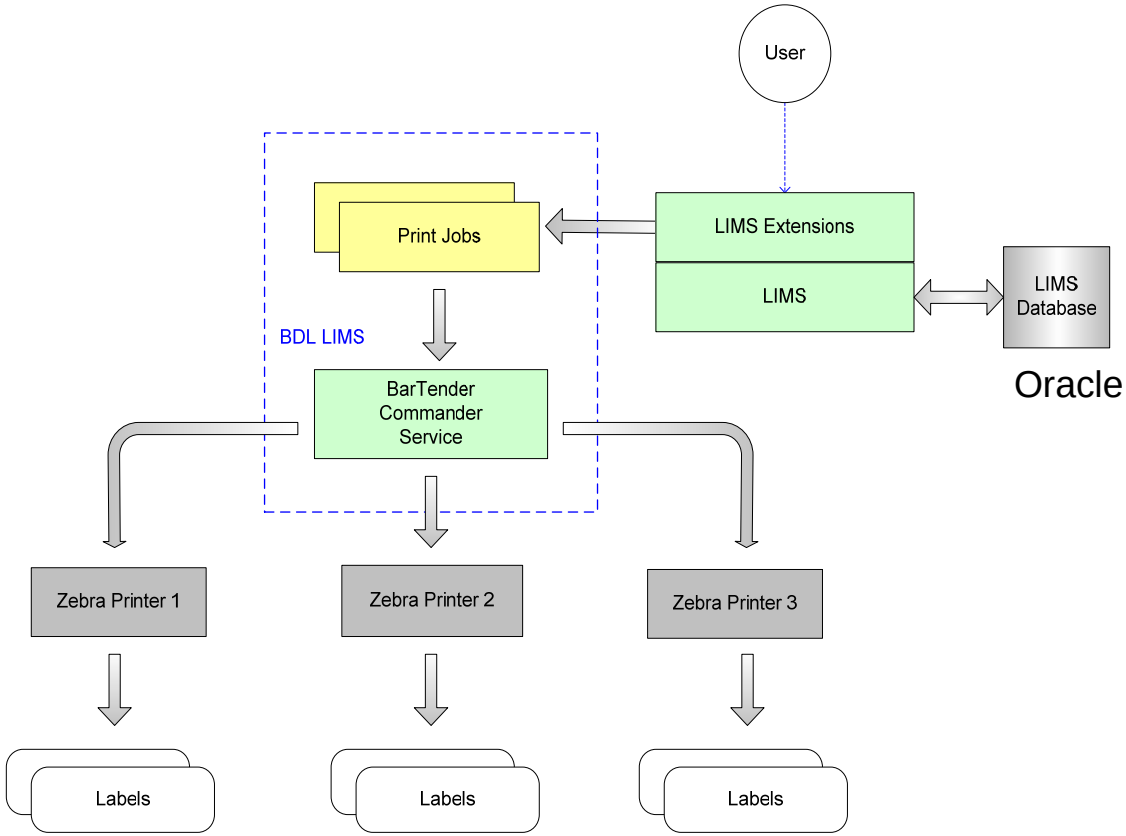
LIMS Barcode Label System v2.0

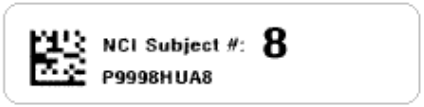


Oracle based

Two-dimensional barcodes

Acknowledgment to Mark Cosentino, SAIC

Subject Label



Draw Label
(5 per subject)

Frozen Aliquot Label
(5-9 per draw)

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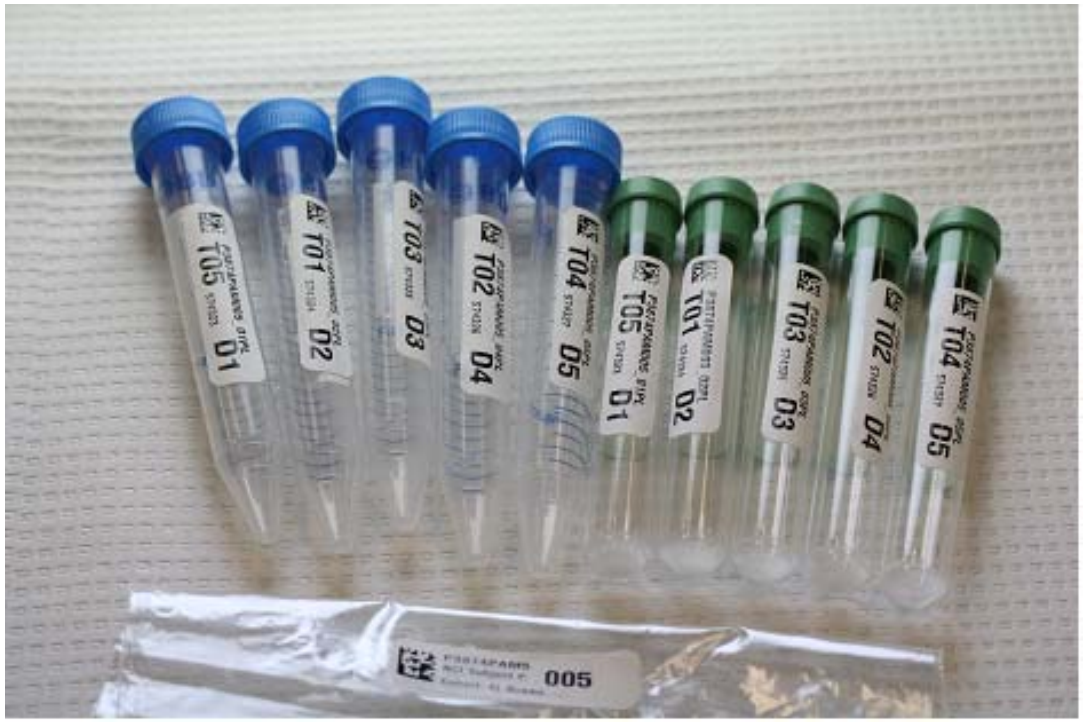
Tube Labeling and Kit Preparation



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

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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

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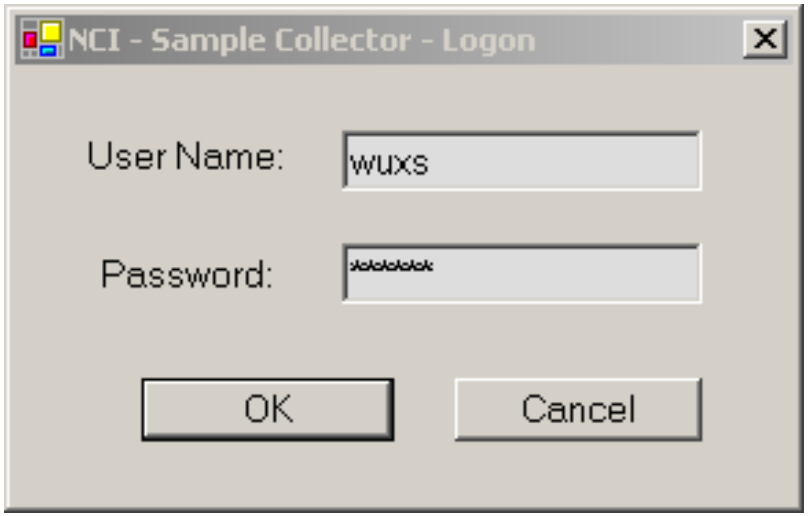
Caprion Proteomics
SAIC/NCI Biospecimen Integrity Project –

SCREEN SHOTS for SAMPLE COLLECTOR PROGRAM

Sample Collector – Login



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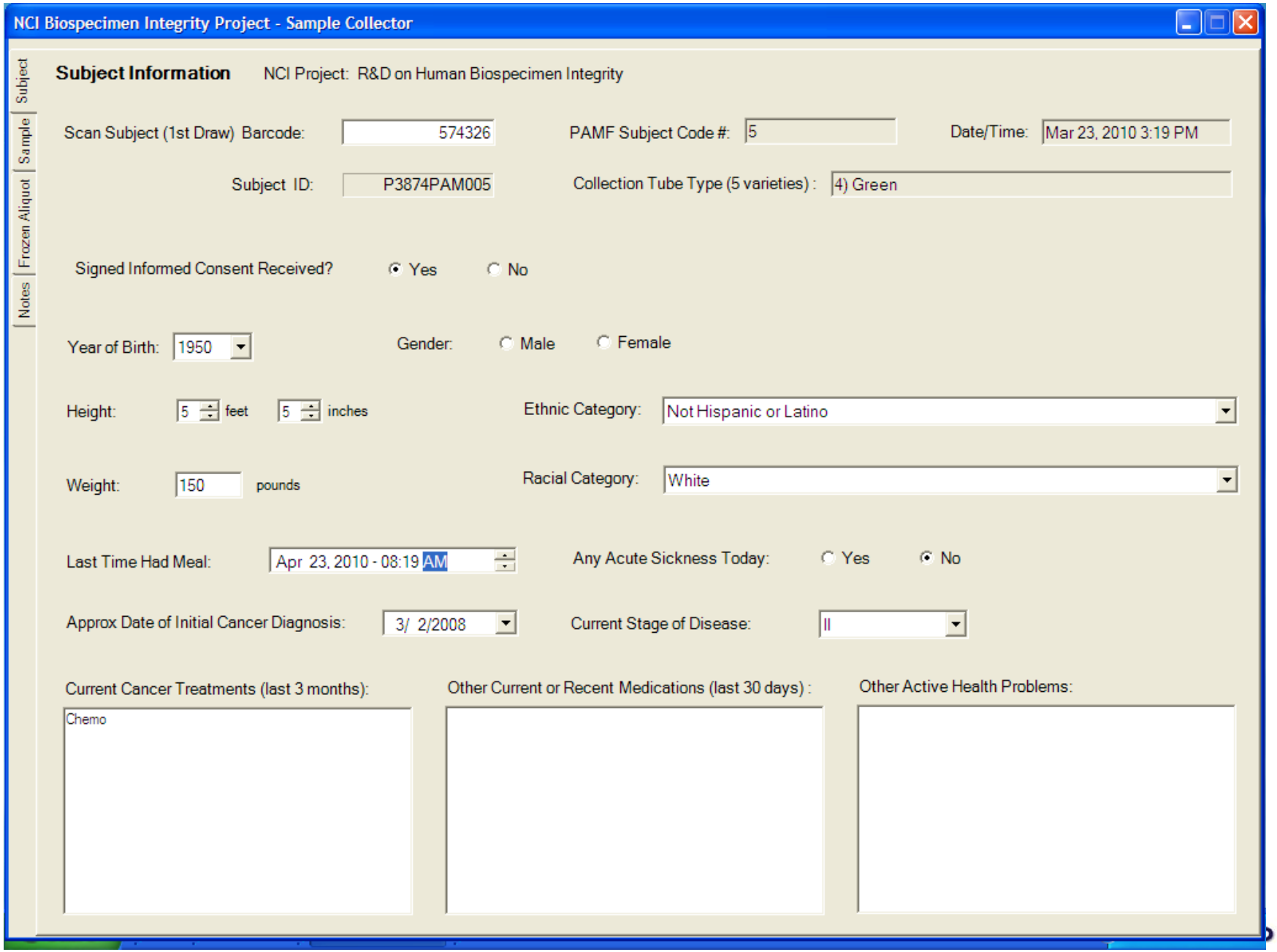
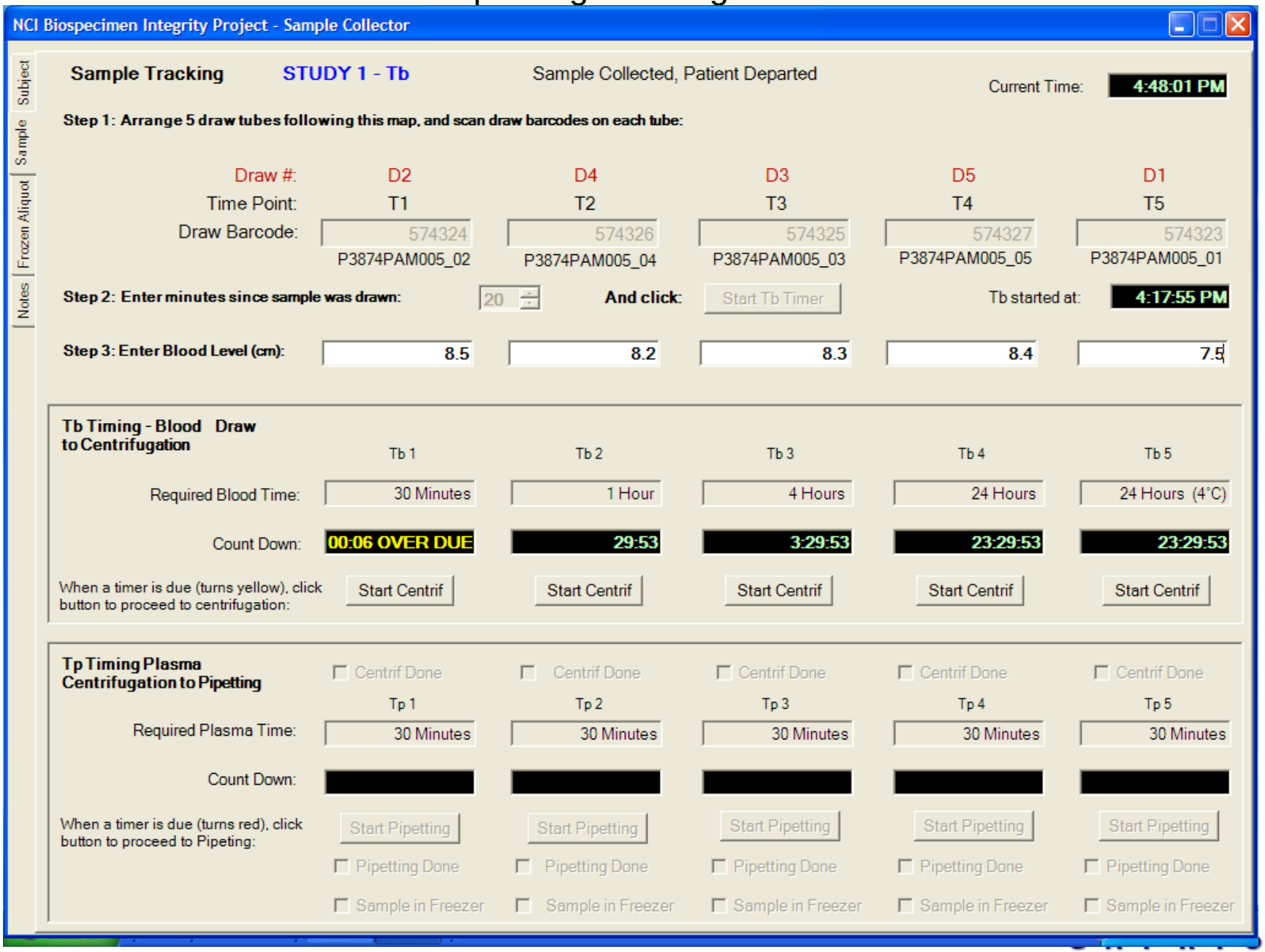
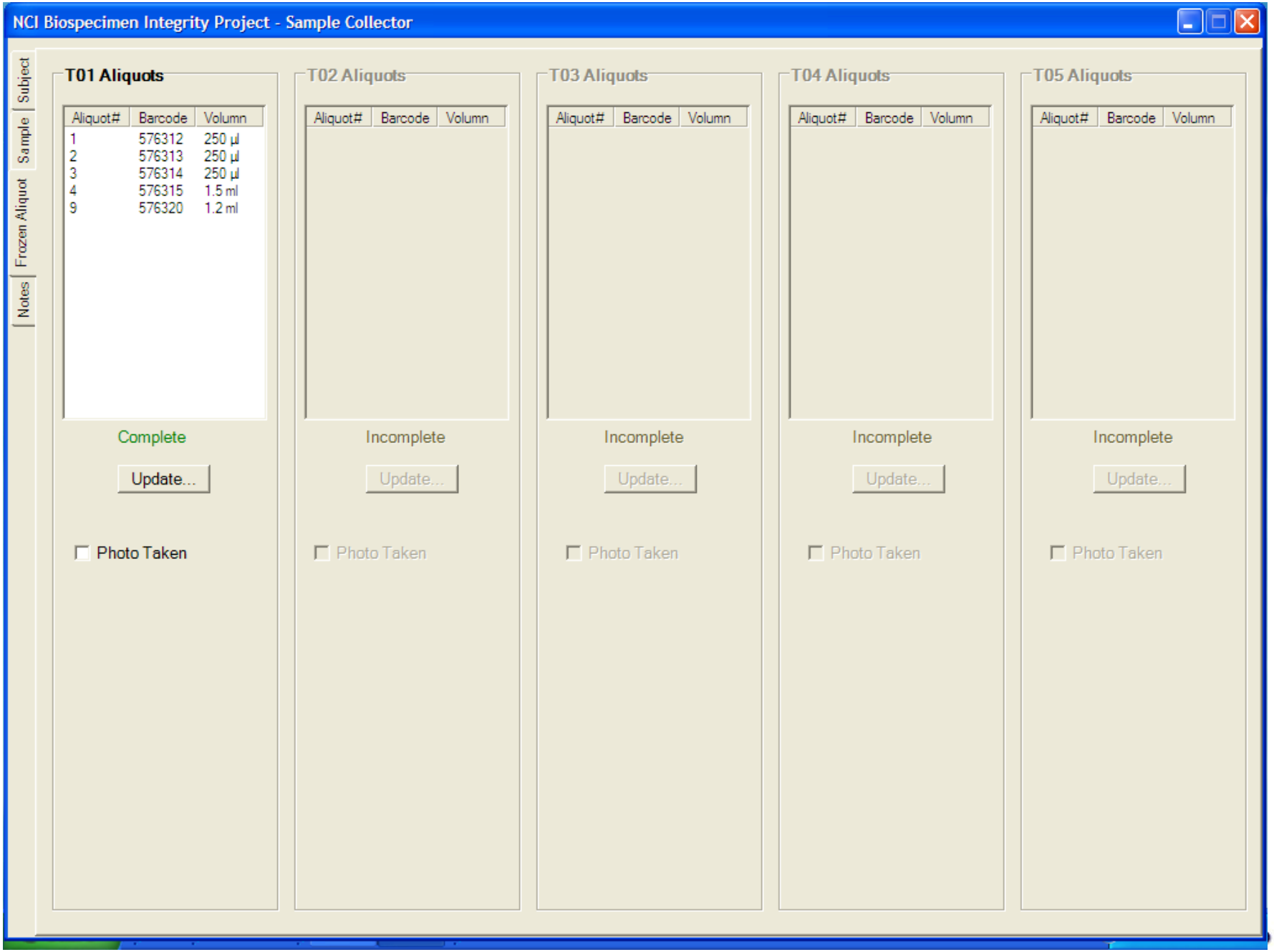
NCI Biospecimen Integrity Project – Sample Collector – Subject Page/Tab



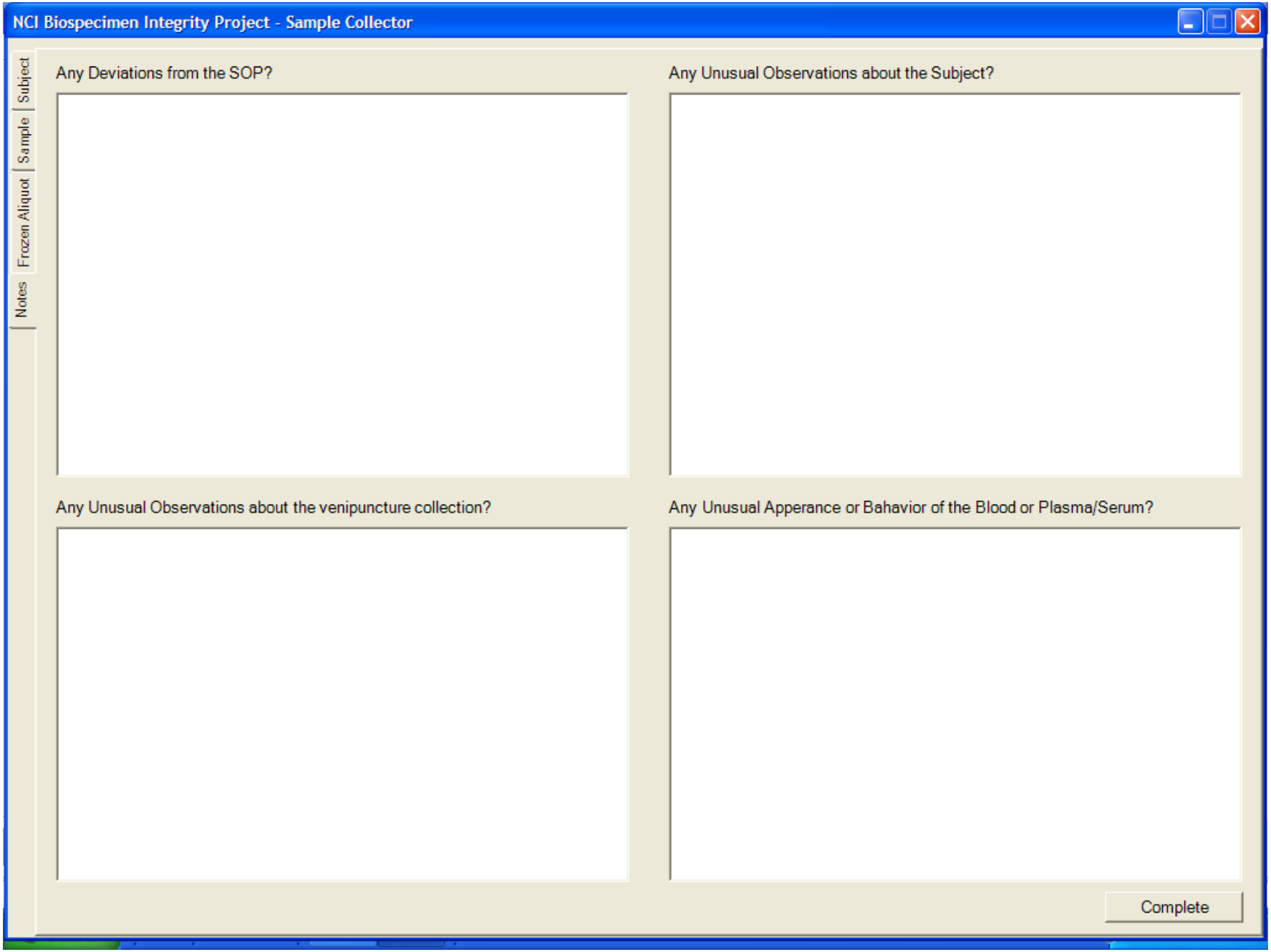
Sample Page – Tb 1 Time Due, Timer and Button Turns Yellow, Flashes and Emits Repeating Warning Noise



Fills in “Aliquots” Page



“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 Apperance or Bahavior 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

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END