Credentialing Plasma and Serum Biospecimen Banks for Proteomics Analyses

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Mass Spectrometry-Based Proteomics Analyses

- **Protein Identification**
  - Posttranslational modifications
- **Protein Quantification**
  - Relative Quantitation
    - Biomarker Discovery
      - Disease specific expression levels
      - Early detection, molecular classification, and diagnosis
    - Protein Interactions
  - Absolute Quantitation
    - Biomarker Verification
    - Protein Modifications

Protein Integrity for Proteomics Analyses

- **Proteolysis**
- **PTMs**
  - Phosphorylation
  - Glycosylation
  - Oxidation
  - Nitration
  - Acetylation
  - Methylation
  - Acylation
  - Sulfation
- **Aggregation and precipitation**

Plasma and serum for biomarker studies

- **Protein and peptide integrity in serum and plasma can be compromised in multiple ways**
  - Artifactual degradation can be a confounding factor in biomarker discovery experiments
- **Determine the effects of specific pre-analytical variables on biospecimen integrity**
  - Assess the impact of preanalytical variables at a global level
  - Sample quality sufficient to yield reproducible, high-quality data
- **Define quality assessment measures for biospecimens used in proteomics workflows**
  - Sensitive markers that can be used as a QC tool to monitor preanalytical variation

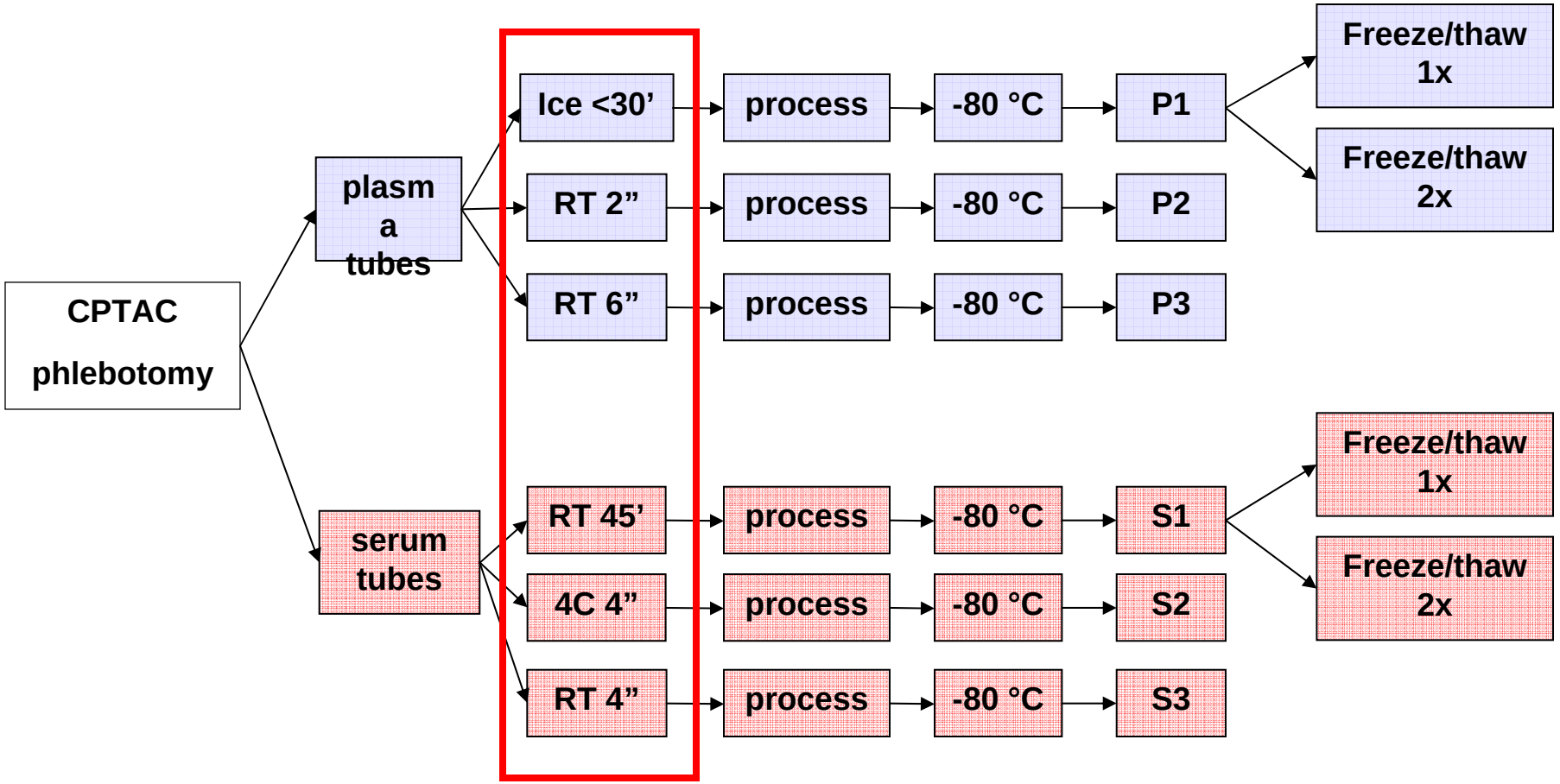
Strategy

- **Generate a defined set of serum and plasma collected and processed under well defined, well controlled conditions**
  - Blood collection using CPTAC protocol
  - Processing using CPTAC (plasma) and EDRN (serum) SOPs
  - Training set
    - Optimally processed samples
    - Processing and storage variables: time, temperature, freeze-thaw cycles
- **Establish ranges for candidate markers of protein damage**
  - Values observed for optimally processed specimens vs. variables
  - Measure in banked plasma and serum samples
- **Establish a panel of reference markers**

Pre-analytical Variables in Blood Collection and Processing

- **Inter-individual and intra-individual variation**
  - Age, gender, history, genetics
- **Venipuncture**
  - Needle gauge, butterfly needle, tubing, adapter type
- **Phlebotomy**
  - Tourniquet technique
  - Patient position, arm position
  - Tube order- first *vs* last, discard tube
- **Collection device**
  - Gel or non-gel separator tube
  - Tube additives, e.g. anti-coagulants or clot activator
  - Manufacturer & device information
  - Tube temperature
- **Blood processing**
  - Time and temperature prior to centrifugation
  - Centrifugation: speed, duration, temperature
  - Protocol for separation of blood from cells
  - Length of time before freezing
- **Storage**
  - Frozen before analysis: snap-frozen, slowly cooled
  - Storage temperature
  - Storage time prior to analysis
  - Number of freeze/thaw cycles

Blood Processing Workflow

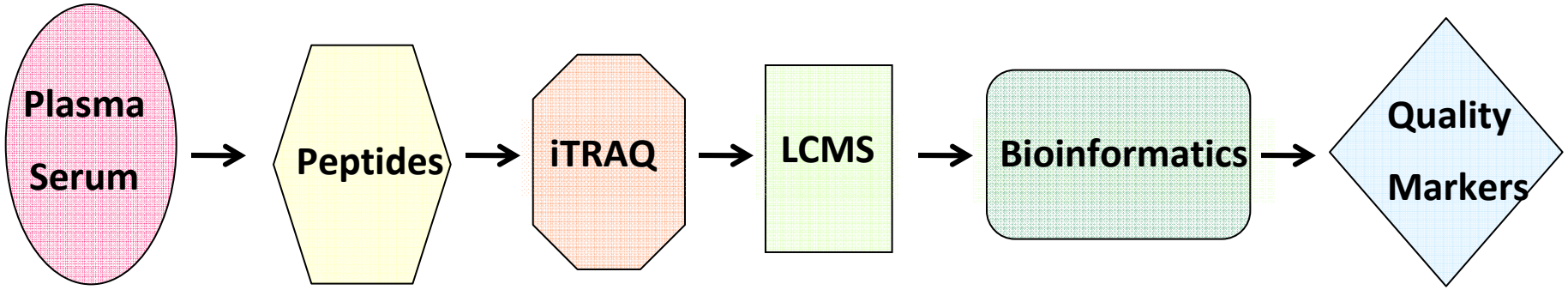


Quantitative Measures of Protein Integrity

- **MS-based proteomics strategy**
  - Identify products of *ex vivo* proteolytic degradation
- **ELISA assays**
  - Quantify the levels of protein oxidation and nitration modifications
- **Size Exclusion Chromatography**
  - Quantify the extent of sample aggregation

Comparison to optimally processed samples

Quantitative Measure of Proteolysis
iTRAQ Labeling Workflow



- Multiplexed assay: 8 samples
- Peptide identification
- Relative quantitation
- Unbiased, non-targeted strategy

Protein Oxidation and Nitration

- **Oxidative stress can result in the formation of reactive oxygen and nitrogen species**
  - Modify protein amino acid side chains
  - Alter protein's structure and/or aggregation state, turnover rates, activity, and protein interaction networks
- **Oxidative stress is known to increase markedly in cancer, diabetes, heart disease, and neurodegenerative diseases.**
- **Oxidized proteins can be used as specific biomarkers of disease.**
- **Sample workup or storage methods can introduce artifactual oxidative modifications through exposure to dissolved oxygen, high or low pH, and/or trace metals.**

Quantitative Measures of Oxidation

- **OxyELISA (Millipore)**
  - Oxygen free radicals and other reactive species introduce carbonyl groups into proteins.
  - Formation of carbonyl groups on protein amino acid side chains is one of the early markers for protein oxidation
  - Quantification of carbonyl groups following derivatization with 2,4-dinitrophenylhydrazine
- Lower limit of sensitivity is 0.2 nmol carbonyl/mg protein
- Intra-assay reproducibility CV < 9%
- Inter-assay of < 17%.

Quantitative Measures of Nitration

- **Nitrotyrosine ELISA (Northwest Life Science Specialties)**
  - Reactive nitrogen species (RNS) can be formed from nitric oxide, hydrogen peroxide, and other pro-oxidants
  - RNS can target tyrosine residues in proteins to form 3-nitrotyrosine adducts
    - A sandwich ELISA using a plate bound capture antibody (anti nitrated KLH) to nitrotyrosine and a biotinylated secondary tracer antibody
    - Lower limit of detection is 2 nM
    - Intra-assay reproducibility CV < 8%
    - Inter-assay of < 8%.

Quantitative Measures of Aggregation

- **Size Exclusion Chromatography with UV detection**
  - Measure the fraction of protein aggregates present in the total protein content of plasma and serum
  - The SEC column functions as a molecular sieve that separates species by size
    - Column with very high M_r exclusion limit ($4x10^7$) to accommodate very large protein aggregates.
    - Measure “aggregate percentage value” for each sample: the ratio between the void volume peak area and total area under all peaks

Statistical Analysis

- **Quantify effects of pre-analytical variables**
  - iTRAQ data (ratio)
  - Oxidation assay (nM)
  - Nitration assay (nM)
  - SEC data (ratio)
- **For each of these four outcomes there are two main goals:**
  - Quantify the effect of procedural variables
  - Describe a normative distribution for a given sample handling procedure for quality assurance use in existing banks
- **Exploratory aim to discover a "signature" combination of peptide quantities that indicates degradation (proteolysis)**

Credentialing Plasma and Serum Biospecimen Banks

- **Plasma**
  - Colorectal cancer
  - Jim Ayers Institute for Precancer Detection and Diagnosis at Vanderbilt University
- **Serum**
  - Breast cancer
  - Early Detection Research Network sample bank at the UCSF Helen Diller Family Comprehensive Cancer Center

Clinical Translational Science Institute at UCSF

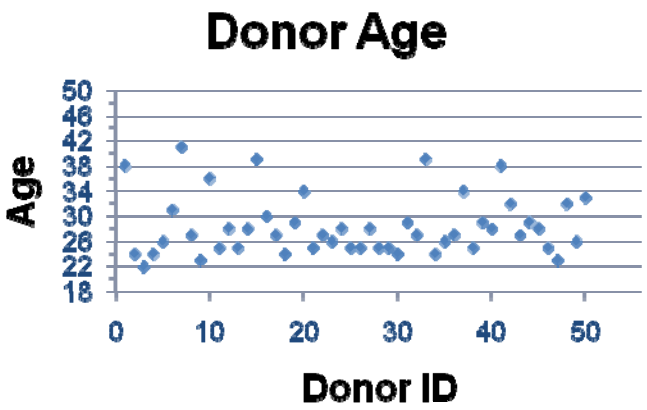
- **Clinical Research Center- CRC**
  - Inpatient and outpatient services
  - Nursing services
  - Bionutrition
  - Sample processing
- **Consultation Services**
  - Biostatistics
  - Study design and Implemenation
  - Data management
  - Ethical Issues
- **Training**
- **Working with industry and community partners**

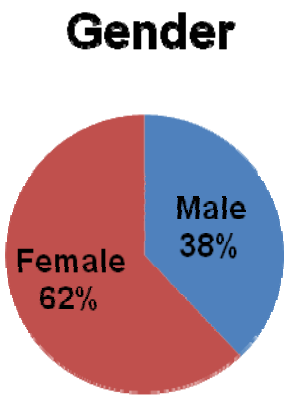
Clinical Research Center Process

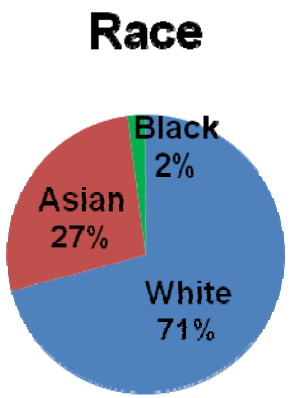
- **CRC Application**
  - Study protocol and services requested
  - CHR /IRB approval
- **CRC Advisory Committee Approval**
- **Meet with CRC nursing staff**
- **Study Implementation forms**
  - MD Orders
  - Consent
  - Inclusion/Exclusion criteria
  - HIPAA
- **Blood draw**
- **Processing**

Collection Statistics

- **52 subjects completed**
  - 44 samples suitable for analysis
    - Plasma collected before serum
    - Sample yield low
    - Samples not processed according to SOP



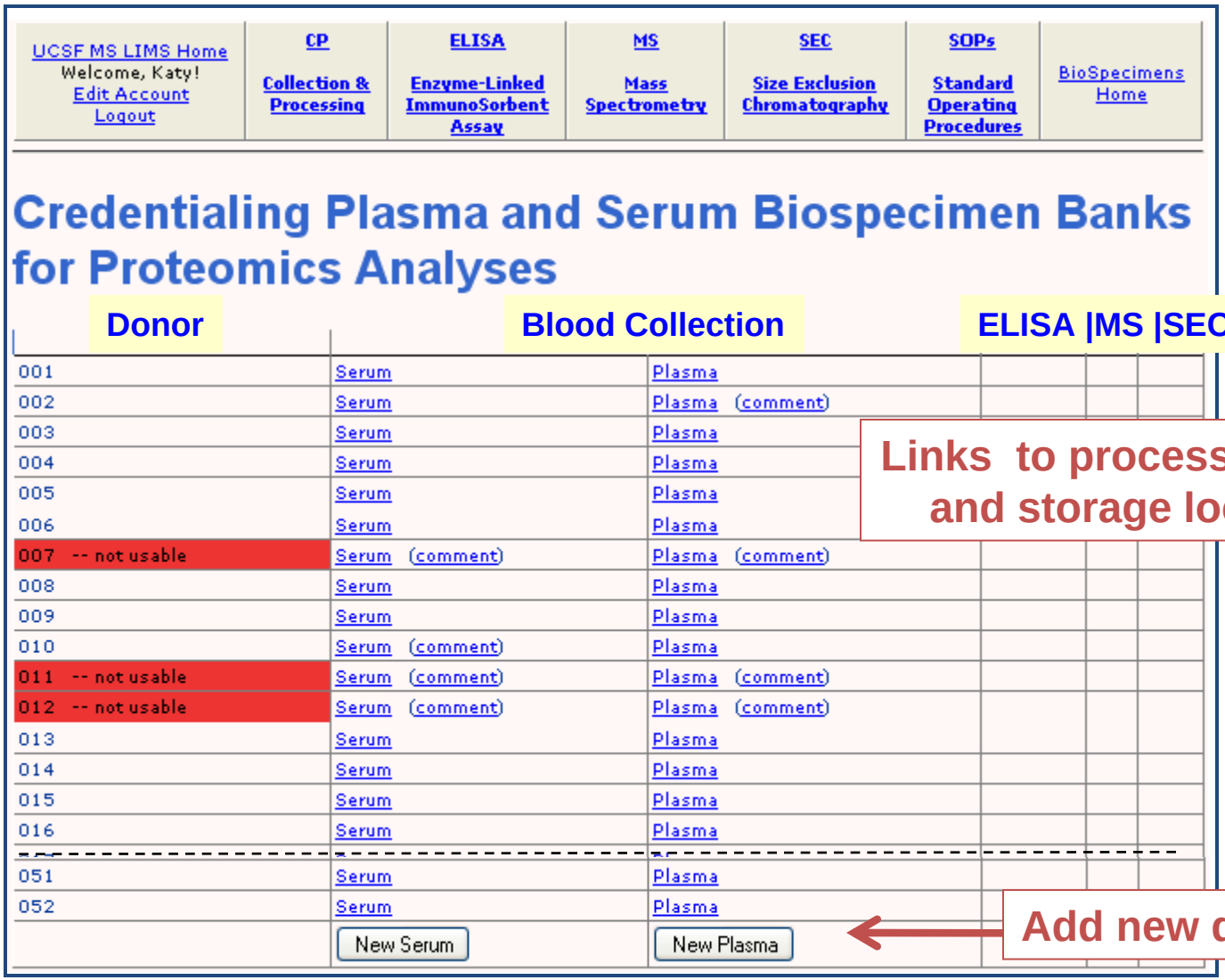




Biospecimens Database

- **mySQL-backed web-accessible electronic information system**
- **Tiered sectors**
  - Blood collection and processing, including sample tracking
  - Analytical data
  - SOPs, audit reports
  - Training records
  - Data reports
- **Password protected, limited access**
- **Servers in a controlled access high security climate controlled room located in the UCSF Library and Center for Knowledge Management**

Biospecimens Database



UCSF MS LIMS Home
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CP — Collection & Processing | ELISA — Enzyme-Linked ImmunoSorbent Assay | MS — Mass Spectrometry | SEC — Size Exclusion Chromatography | SOPs — Standard Operating Procedures | BioSpecimens Home

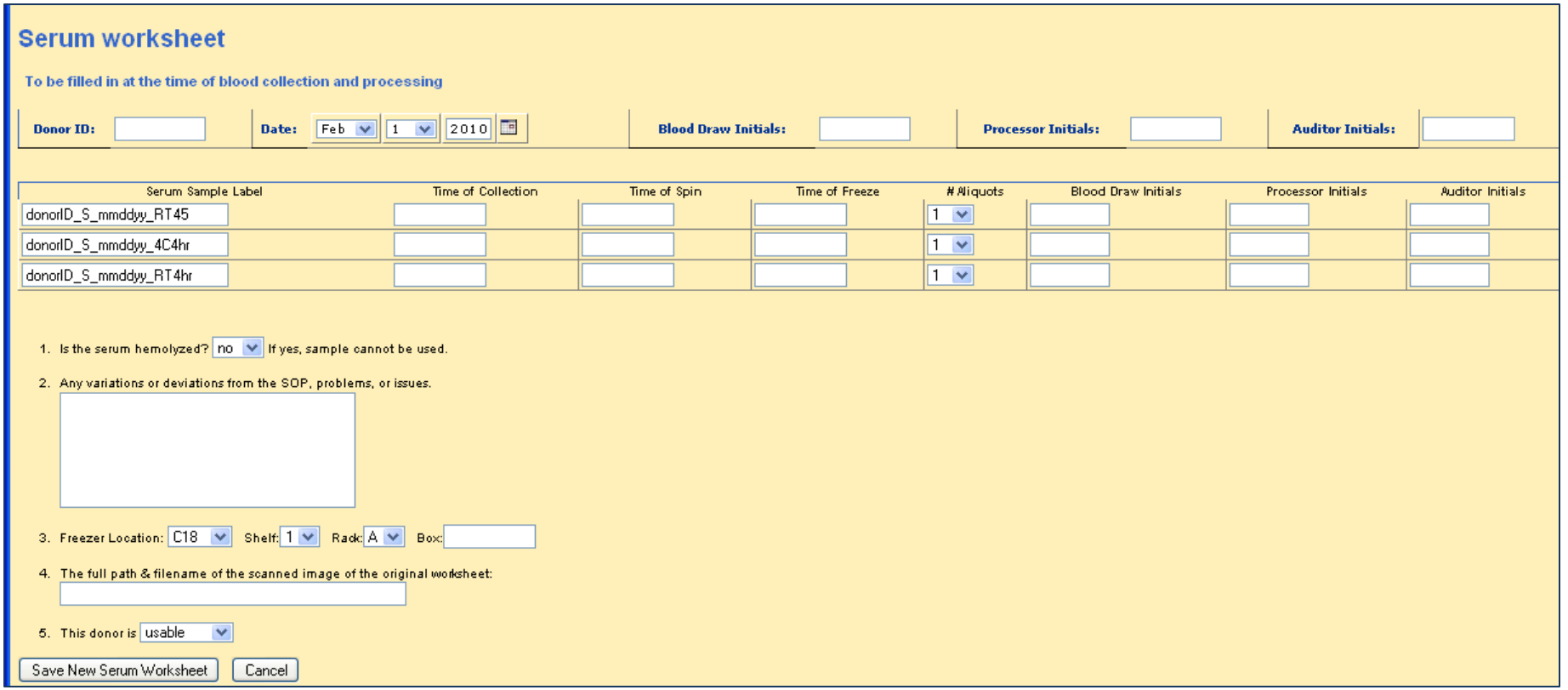
Credentialing Plasma and Serum Biospecimen Banks for Proteomics Analyses

Donor	Blood Collection		ELISA |MS |SEC		
001	Serum	Plasma			
002	Serum	Plasma (comment)			
003	Serum	Plasma			
004	Serum	Plasma			
005	Serum	Plasma			
006	Serum	Plasma			
007 -- not usable	Serum (comment)	Plasma (comment)			
008	Serum	Plasma			
009	Serum	Plasma			
010	Serum (comment)	Plasma			
011 -- not usable	Serum (comment)	Plasma (comment)			
012 -- not usable	Serum (comment)	Plasma (comment)			
013	Serum	Plasma			
014	Serum	Plasma			
015	Serum	Plasma			
016	Serum	Plasma			
051	Serum	Plasma			
052	Serum	Plasma			
	New Serum	New Plasma			

Links to processing data and storage location

Add new data

Serum worksheet



Serum worksheet

To be filled in at the time of blood collection and processing

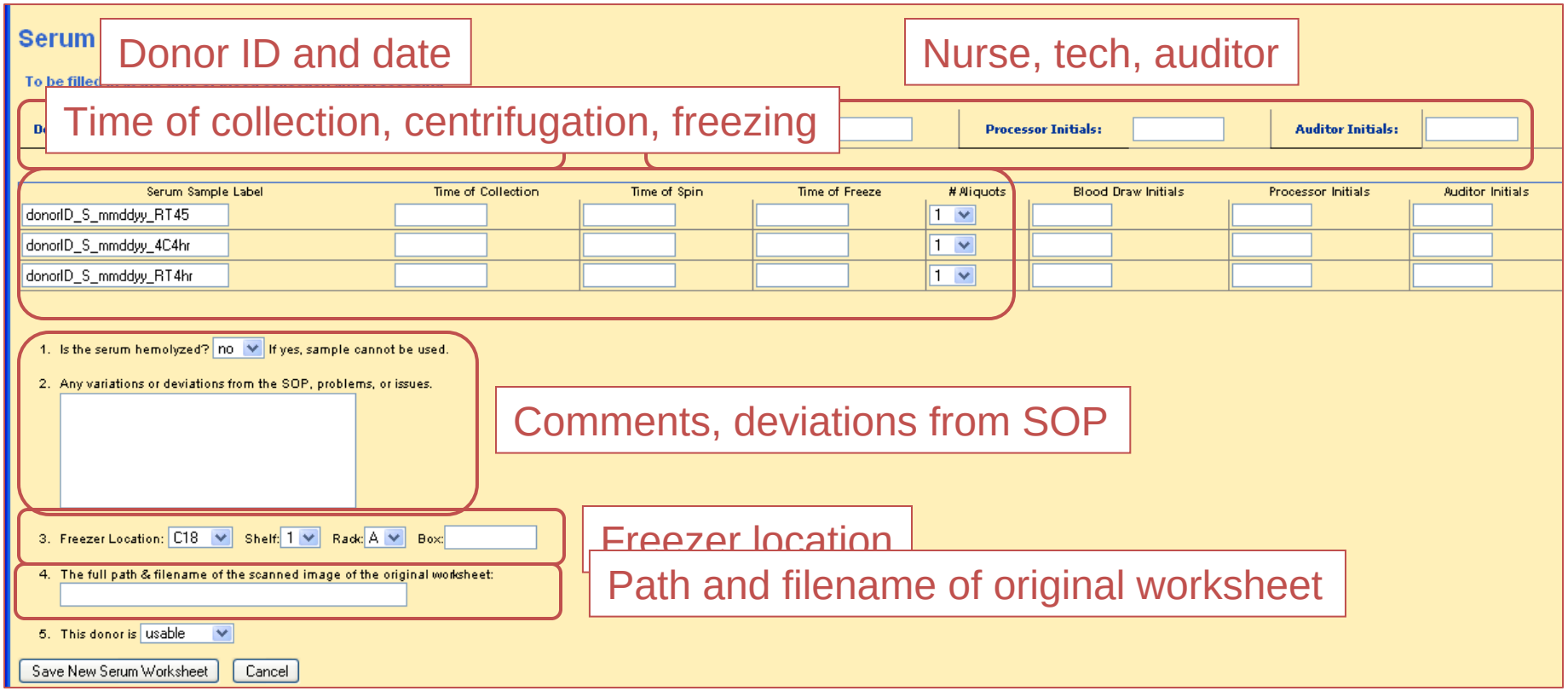
Donor ID: | **Date:** Feb 1 2010 | **Blood Draw Initials:** | **Processor Initials:** | **Auditor Initials:**

Serum Sample Label	Time of Collection	Time of Spin	Time of Freeze	# Aliquots	Blood Draw Initials	Processor Initials	Auditor Initials
donorID_S_mmddyy_RT45				1			
donorID_S_mmddyy_4C4hr				1			
donorID_S_mmddyy_RT4hr				1			

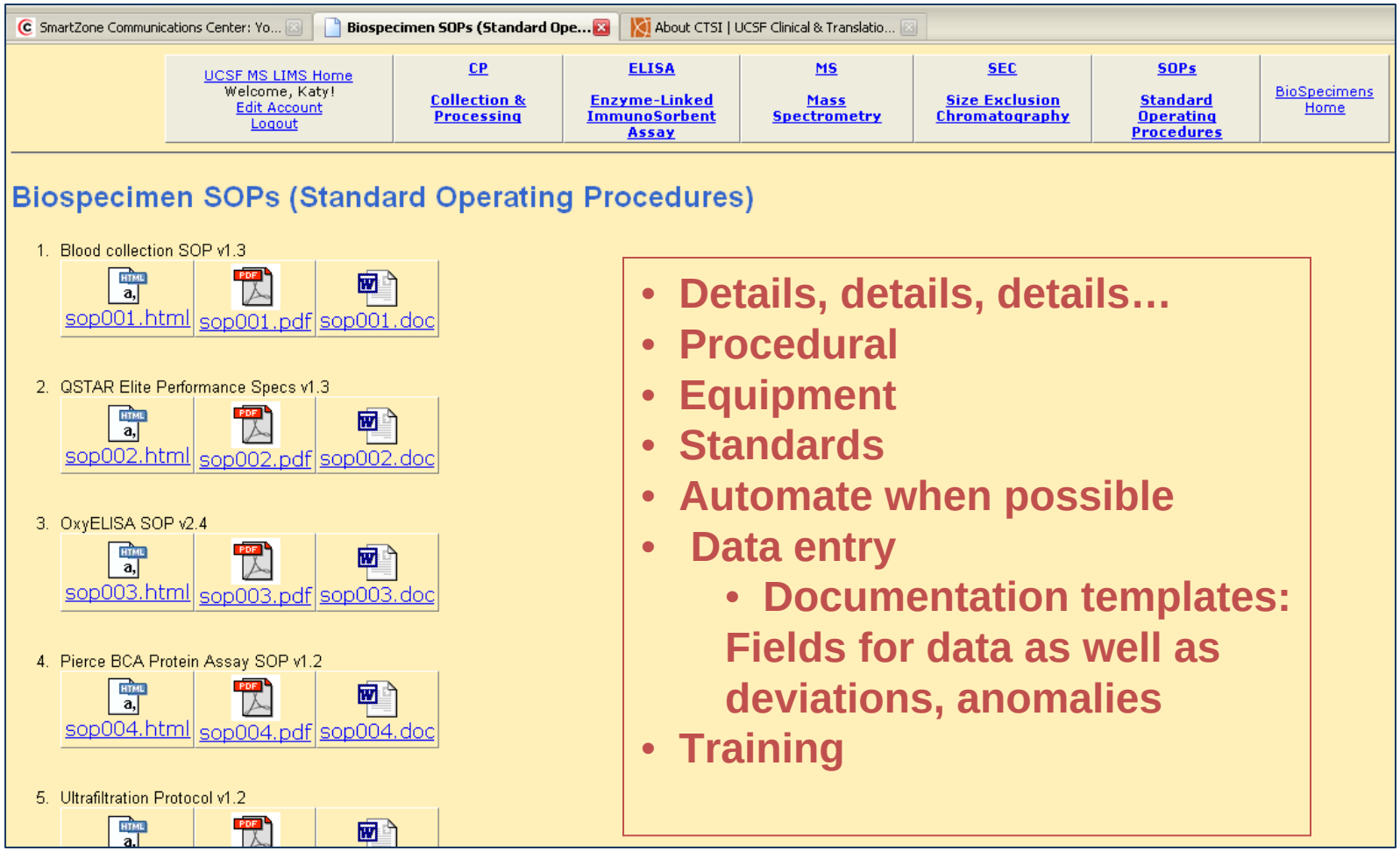
1. Is the serum hemolyzed? no If yes, sample cannot be used.
2. Any variations or deviations from the SOP, problems, or issues.
3. Freezer Location: C18 Shelf: 1 Rack: A Box:
4. The full path & filename of the scanned image of the original worksheet:
5. This donor is usable

Save New Serum Worksheet | Cancel

Serum Collection and Processing Data



SOPs



- Details, details, details…
- Procedural
- Equipment
- Standards
- Automate when possible
- Data entry
  - Documentation templates: Fields for data as well as deviations, anomalies
- Training

Outcomes

- **Quantify the effect of procedural variables**
  - Describe a normative distribution for a given sample handling procedure for quality assurance use in existing banks
- **Exploratory aim to discover a “signature" combination of peptide quantities that indicates degradation (proteolysis)**
- **Generate information on inter-individual variability**
  - N=50
- **Generate a model of a proteomics study of cases vs. controls**
  - Optimally processed samples = controls
  - Evaluate statistical approaches

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- **SAIC Frederick**
- **NCI**