

A blue-tinted, close-up photograph of a microarray chip, showing a grid of small, square spots. The spots are arranged in rows and columns, with some numbers visible, such as '2' and '260'. The image is slightly blurred and has a soft, glowing effect.

Bringing Metrology to Clinical Proteomic Research

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Proteomics

- The goal of *proteomics* is to identify and quantify all proteins and protein isoforms in the cells, tissues, and other biosamples from an organism.
- Proteomics research has been split into 2 distinct approaches:
 - Top-down proteomics (intact proteins)
 - Bottom-up proteomics (enzymatically-derived peptides)

Clinical Proteomics

- The goal of *clinical proteomics* is to identify and quantify a human protein or proteins whose change in structure or concentration can be used to diagnose disease.

Clinical Biomarker Pipeline:



Rifai, N., Gillette, MA, and Carr, SA (2006) Nature Biotechnol. 24: 971 - 983

Proteomics Today

- **No performance criteria**
 - Poor confidence in protein measurement results (HUPO PPP: less than 50 % repeatability of protein ID for replicate analysis within the same lab)
 - Difficulty in assessing agreement of different experiments
 - Conflicting reports in the literature
 - Diminishing opportunities for future research and research funding

National Cancer Institute's Clinical Proteomics Technology Assessment for Cancer (CPTAC)

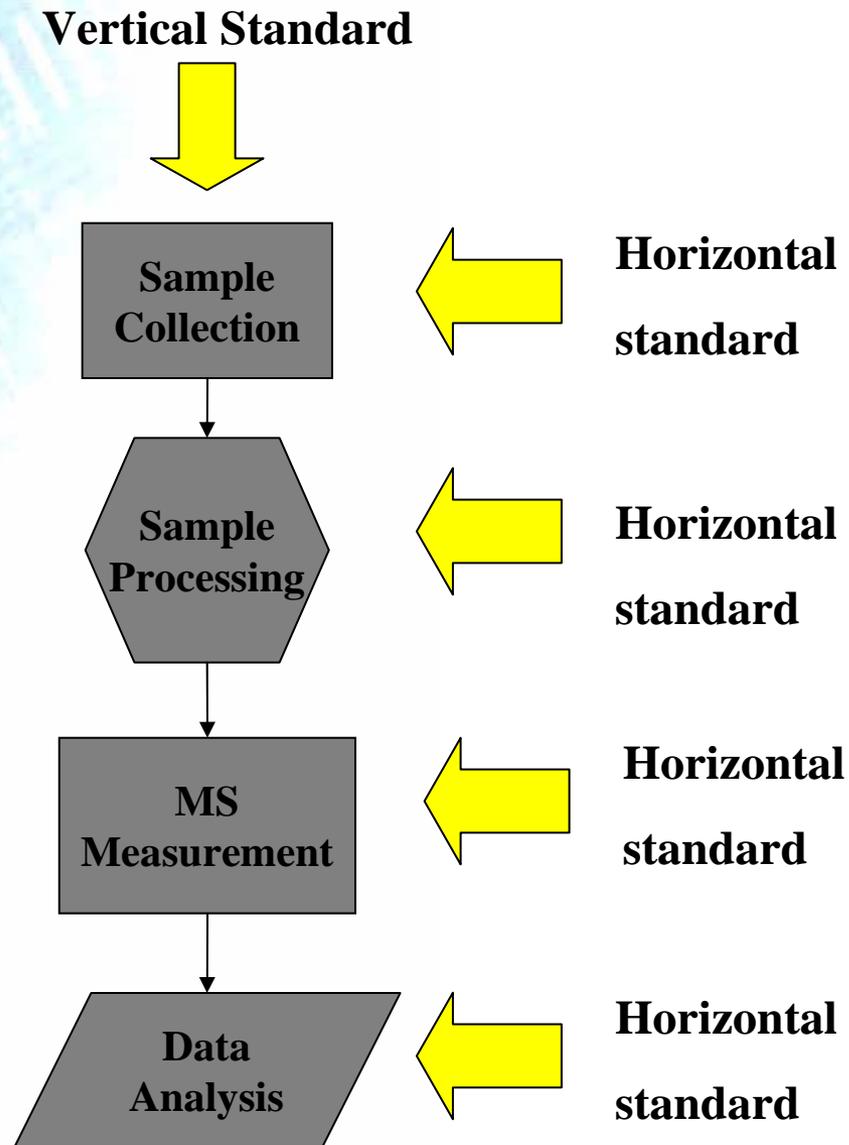
- 5-year funding (starting Sept. '06) for 5 research teams
- Focus on enhancing proteomic measurement capabilities for clinical cancer research with emphasis on mass spectrometry and affinity capture platforms
- Teams participate in inter-laboratory studies aimed at improving reproducibility and comparability of clinical proteomic data
- NIST is providing reference materials for research teams to assess measurement quality through inter-laboratory studies

Clinical Biomarker Pipeline



- Biomarker Discover
 - Qualitative and relative quantification measurements done between normal and disease-state samples
 - Initial work performed in serum/plasma; recent focus has been on tissue
 - LC-MS/MS using data-dependent acquisition
- Biomarker Verification
 - Absolute quantification of “signature peptides” from candidate biomarker proteins
 - LC-MS/MS with multiple-reaction monitoring (MRM)

Horizontal vs. Vertical Reference Standards



NIST Reference Materials for Clinical Proteomics

- SRM 3952
 - Complex mixture of synthetic peptides
 - Horizontal standard
 - Designed for routine QC of MS instrument performance in proteomics
- SRM 3953
 - Yeast lysate protein mixture
 - Vertical standard
 - Designed to validate methodologies in discovery based proteomics using a “reference proteome”

Future NIST Efforts Toward Improving Clinical Proteomic Measurement Quality

- Additional complex-matrix horizontal standards (plasma-based RMs for verification type proteomic studies)
- Measurement tools for assessing the performance of affinity reagents in proteomic arrays
- Development and validation of novel affinity capture reagents