

**Mass Spectrometric Immunoassay
● for the Determination of Clinically
Relevant Target Protein Variants**

Eric E. Niederkofler, Ph. D.

Senior Scientist

ThermoFisher Scientific

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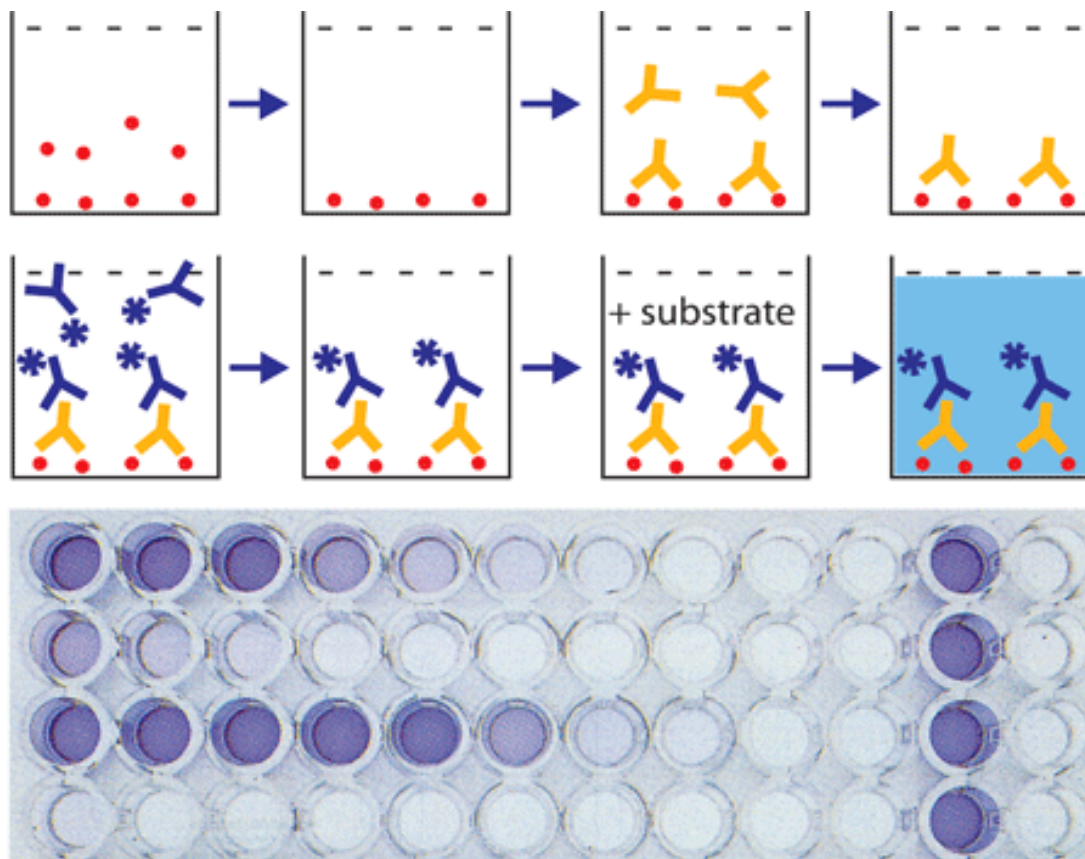
San Jose, CA

Agenda

- Review Classical Immunoassay technologies
- Thermo Scientific MSIA Pipette Tip technologies
- Mass Spectrometric Immunoassay (MSIA)
- Protein Micro - Heterogeneity
- Protein Variants for the Detection of Pre- and Type 2 Diabetes

Conventional Immunoassays

EIA/ELISA



Conventional Immunoassays

EIA/ELISA

Pros:

- Provide concentration values of a target analyte.
- Fast (hours), highly sensitive & reproducible, very easy to use and has an established trust with the clinical community.
- High throughput (96 and 384 formats).
- Data is used as a metric against established concentration levels from historic population analyses.

Cons:

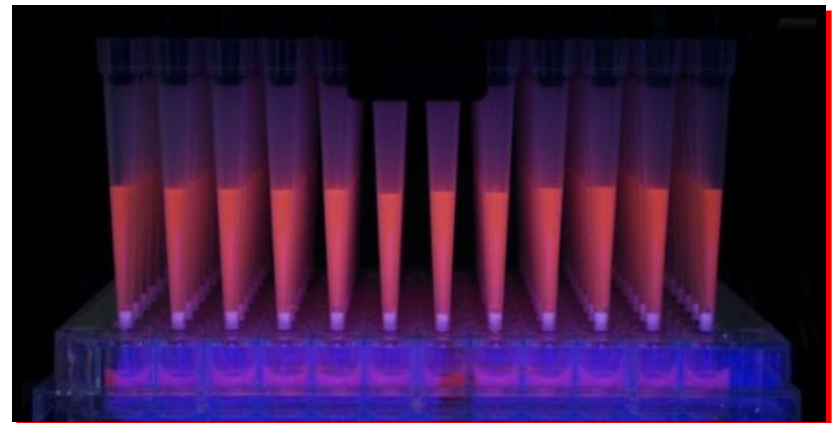
- Is a legacy technology.
- Are blind to heterogeneity if present in the target analyte.

Thermo Scientific MSIA Pipette Tip Technology

Thermo Scientific MSIA Pipette Tips

Novel and proprietary assay format

- Originally developed and demonstrated in 1995 by Intrinsic Bioprobes, Inc.
- Is a simple, yet elegant, approach to micro-scale protein/peptide purification for analytical measurement.
- Original design utilized direct coupling of the immuno-affinity reagent to the affinity support surface, however, next generation technologies utilize universal binding ligands (i.e., Streptavidin, Protein A/G, etc.)

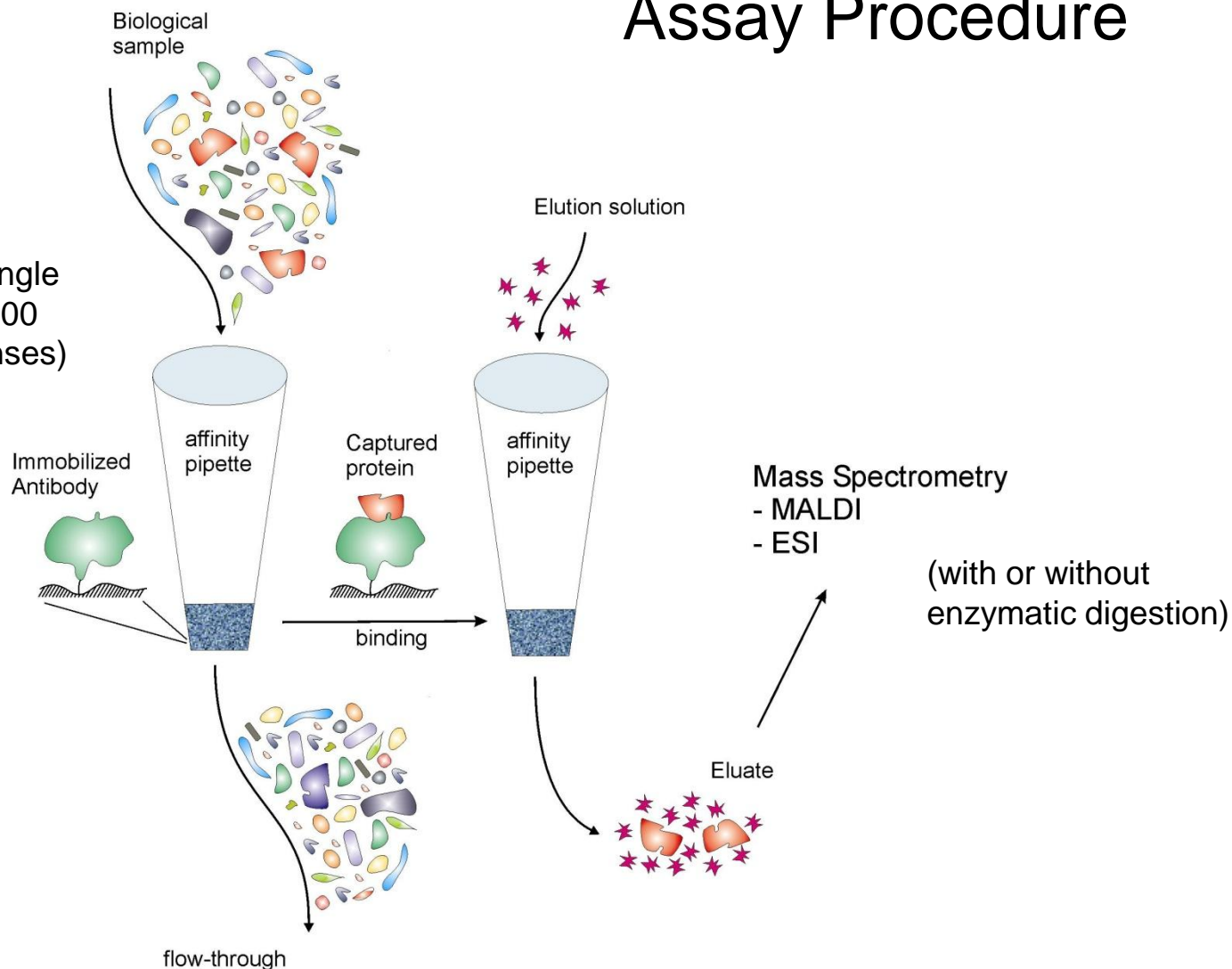


Mass Spectrometric ImmunoAssay (MSIA™)

Technological Advantage – MSIA™ Workflow

Assay Procedure

Repetitive pass of a single sample volume (100-500 aspirations and dispenses)



Technological Advantage – MSIA™ Workflow

Automated Pipetting Workstation Integration



Thermo Scientific Versette Pipetting Workstation



Technological Advantage – MSIA™ Workflow

Integration with Multiple MS Detectors

- Demonstrated with both ESI and MALDI formats

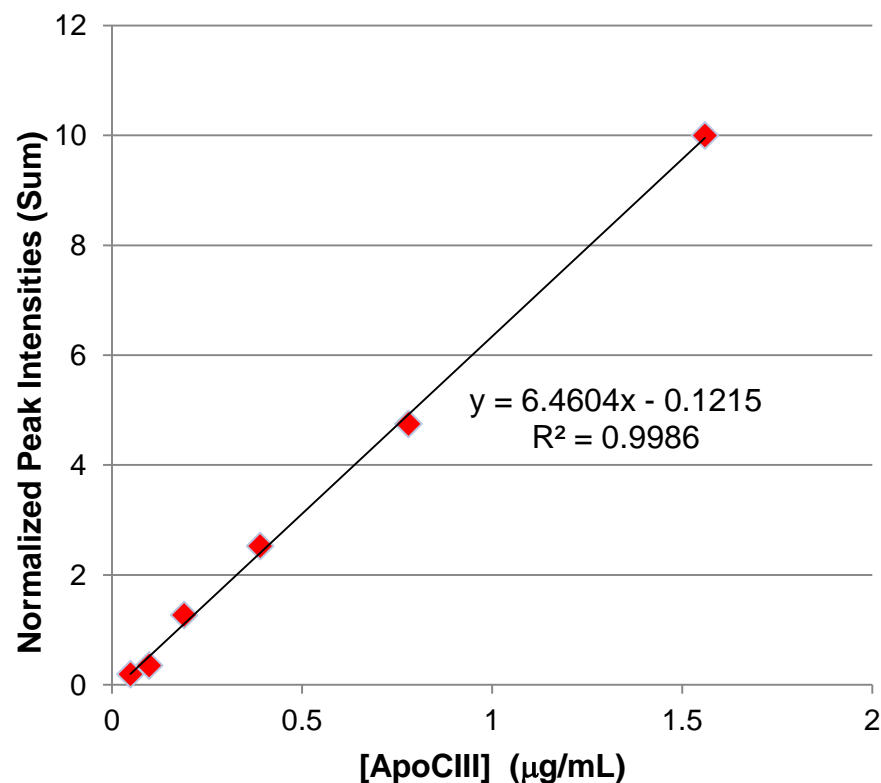
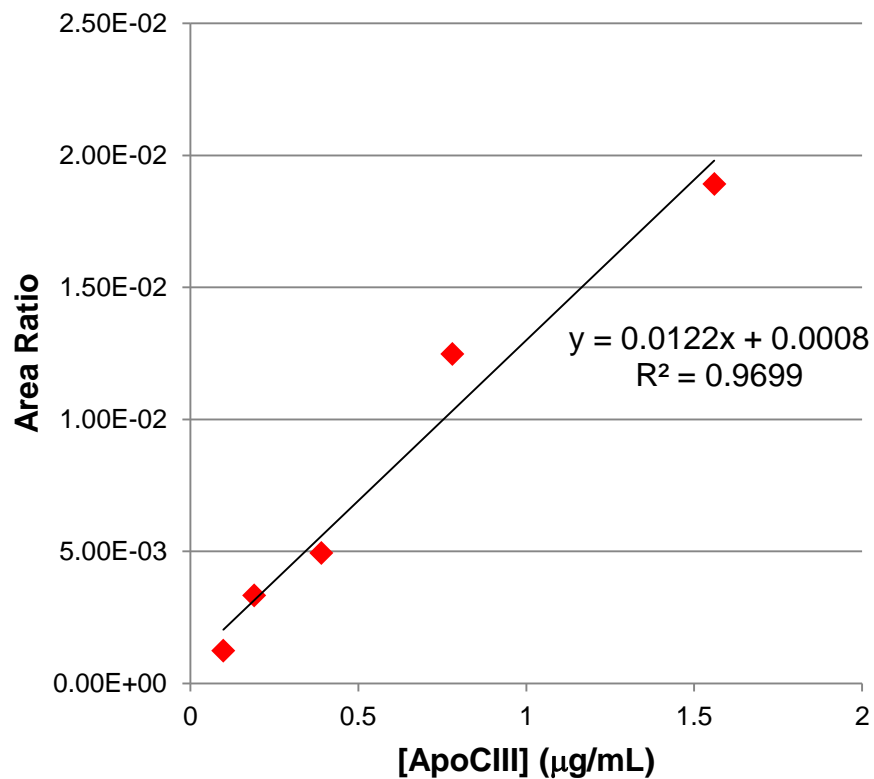


Thermo Scientific TSQ Vantage Triple Stage Quadrupole Mass Spectrometer

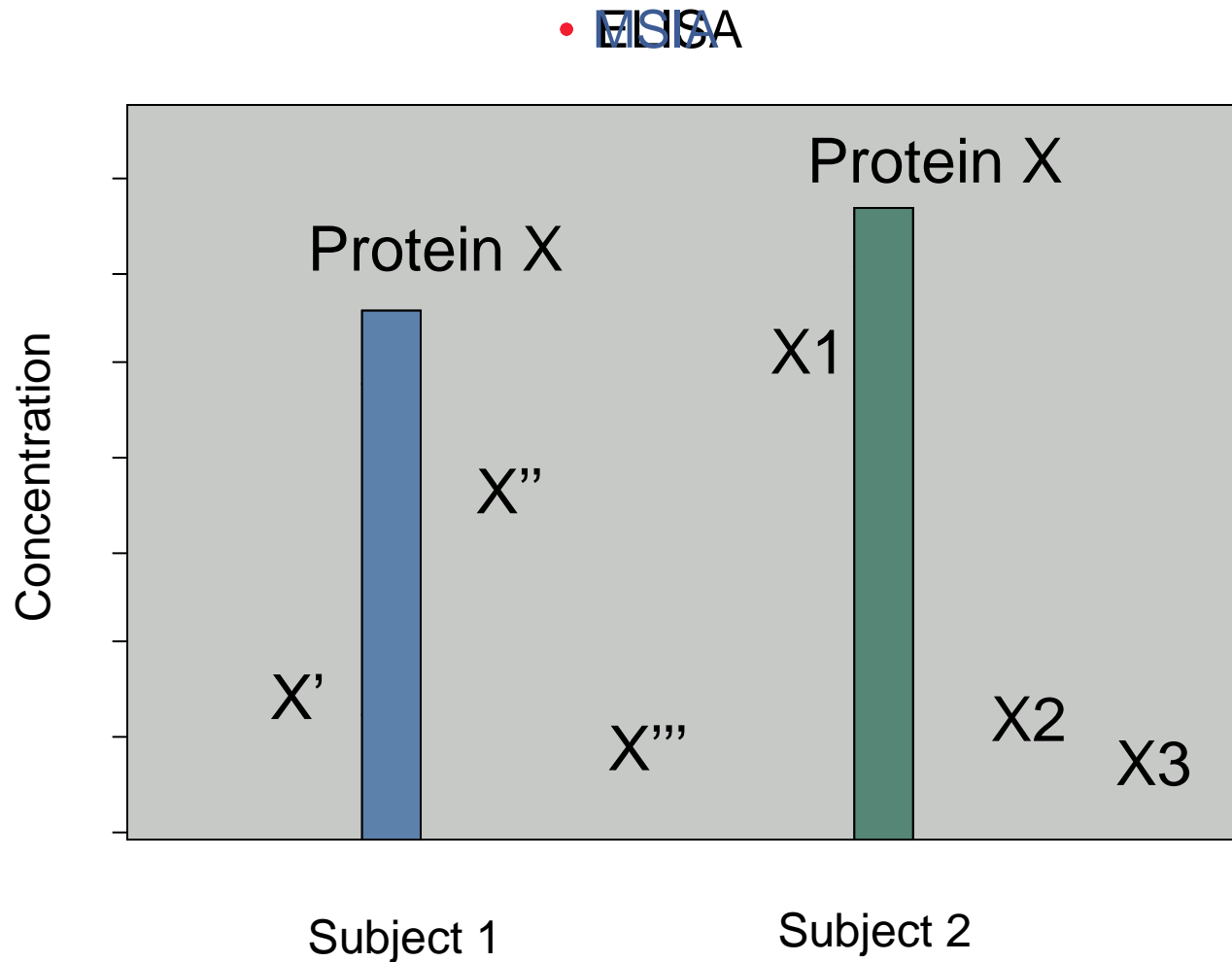
Technological Advantage – MSIA™ Workflow

Quantitative Measurements

- Demonstrated with both ESI and MALDI formats



MSIA key advantage: Protein Micro-Heterogeneity



Biomarker Application (Pre- and Type 2 Diabetes Mellitus)

“MS based approaches for diabetes biomarker discovery”

Grant No. R42DK071290

06/01/05-09/30/09

Targeted Assay

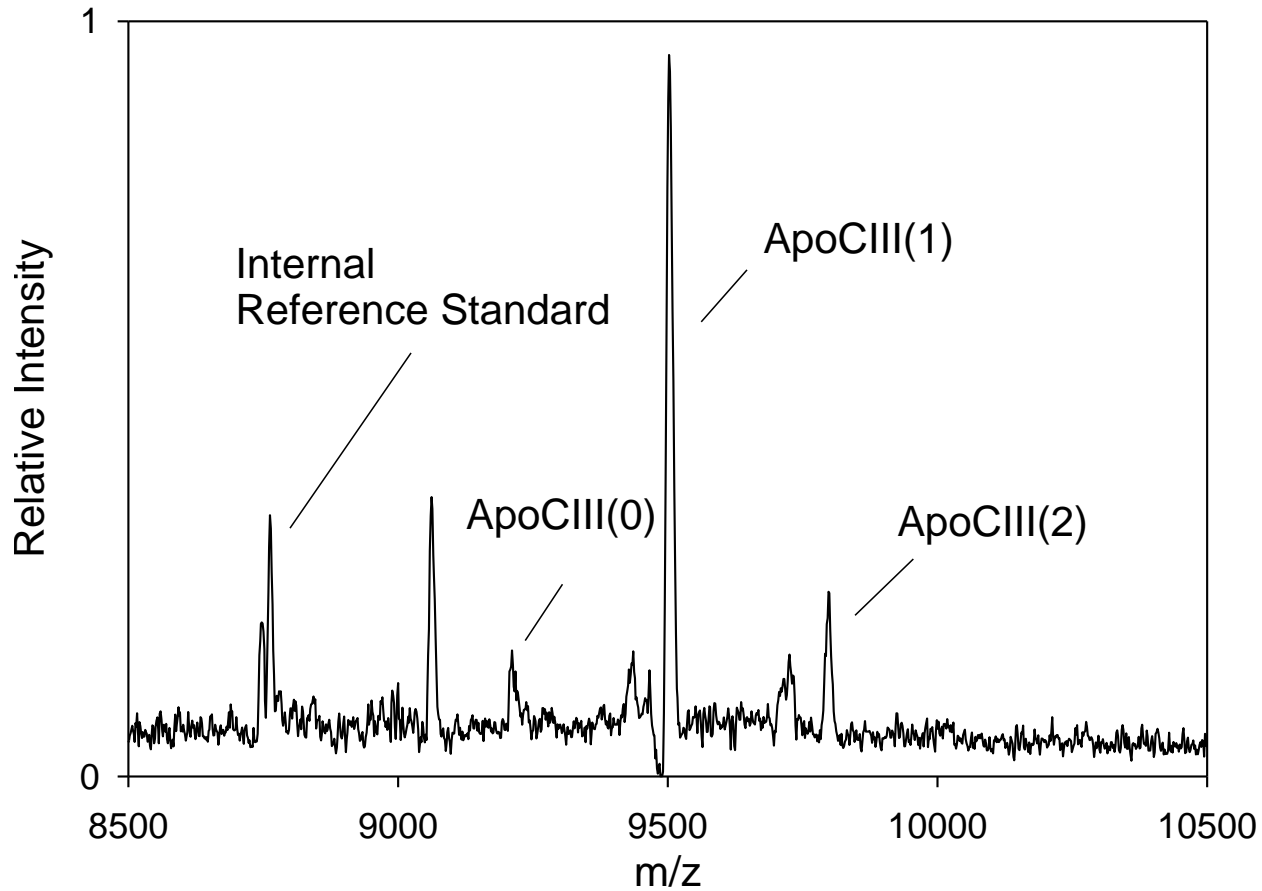
- ApoCIII: expressed as 78 AA protein, MW=9,421.3
- Human plasma concentration: ~ 50 µg/mL
- Antibody: Academy Biomedical (Catalog No. 3GA-G2b)

Semi Quant - Qualitative Analysis

- Combined human plasma sample aliquot with known amount of IRS
- Diluted sample mix with buffer
- Applied automated MSIA technology and acquired MS
- Established integrals for all the ApoCIII isoforms detected within each sample

Qualitative Measurements

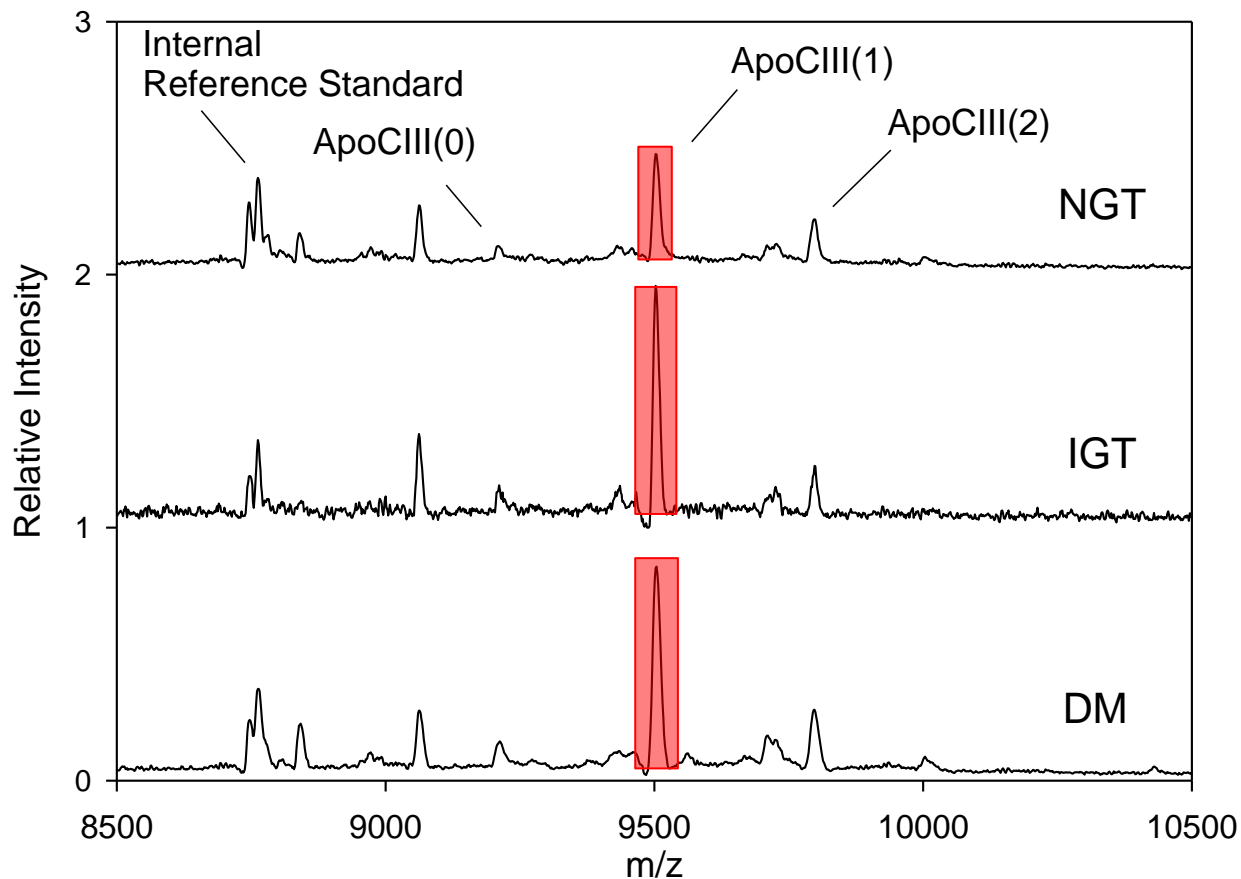
- Differentiation of Mass Shifted Protein Variants



Application - MSIA™

Qualitative Measurements

- Representative MS traces from each sample class



Application - MSIA™

Patient Classification

- Used the normalized ApoCIII(1) levels against the OGTT classification

NGT; n = 235

IGT; n = 96

T2DM; n = 25

IGT T-Test:

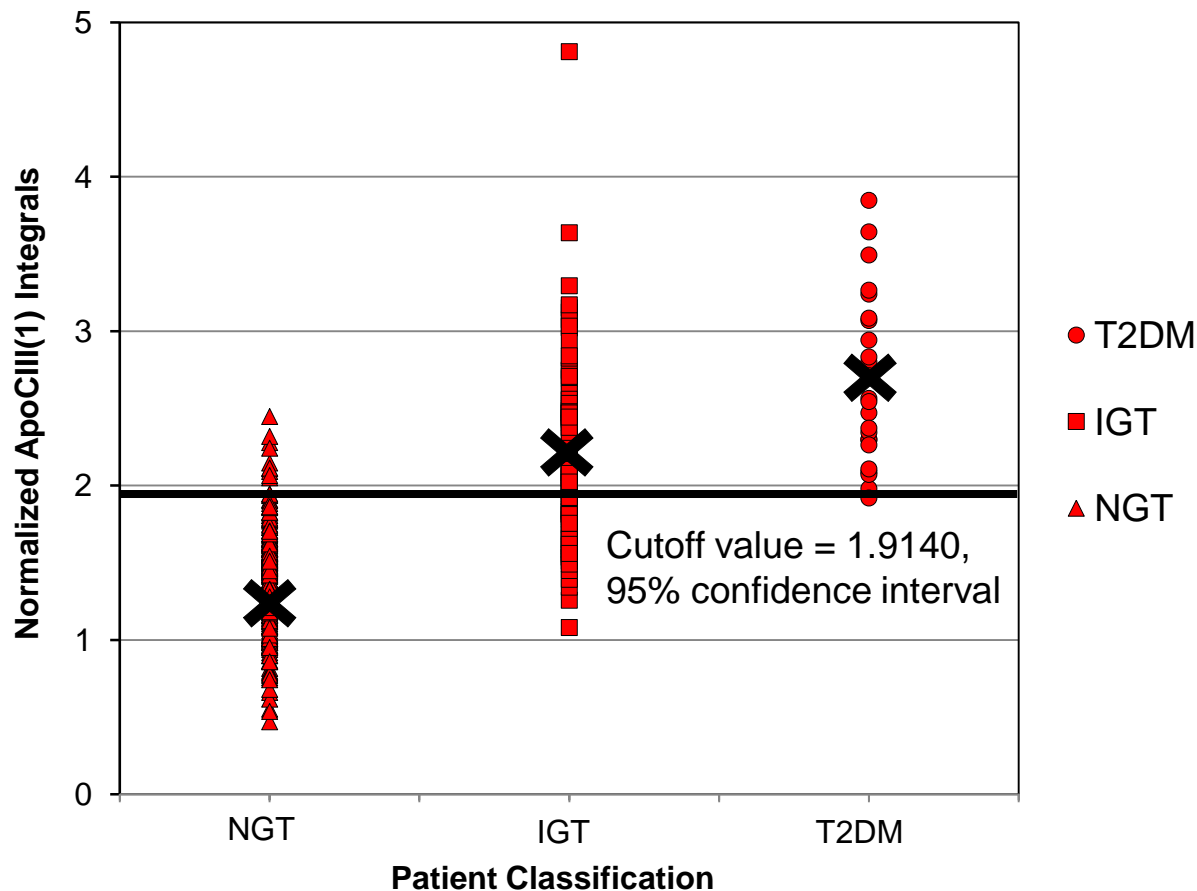
T value = 15.805,

P value < 0.00001

T2DM T-Test:

T value = 16.471,

P value < 0.00001



Application - MSIA™

Patient Classification

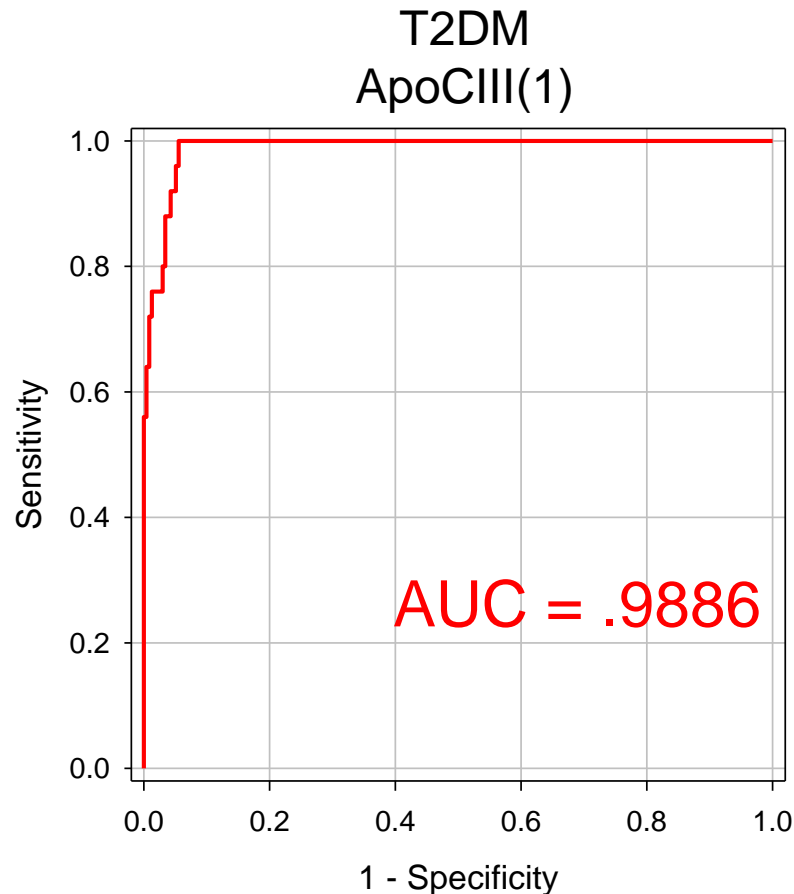
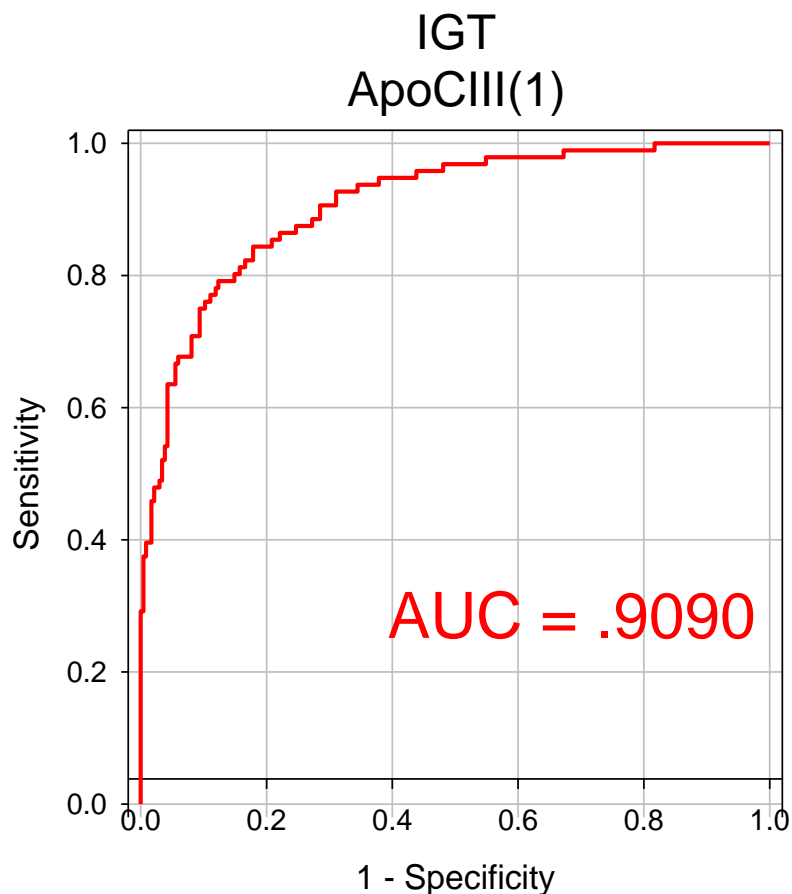
- Clinical Sensitivity and Specificity determined at the 95% confidence interval.

	Sensitivity	Specificity
IGT		
ApoCIII(1)	84.0 %	87.0 %
T2DM		
ApoCIII(1)	100 %	94.5 %

Application - MSIA™

Receiver Operating Characteristic (ROC) curves

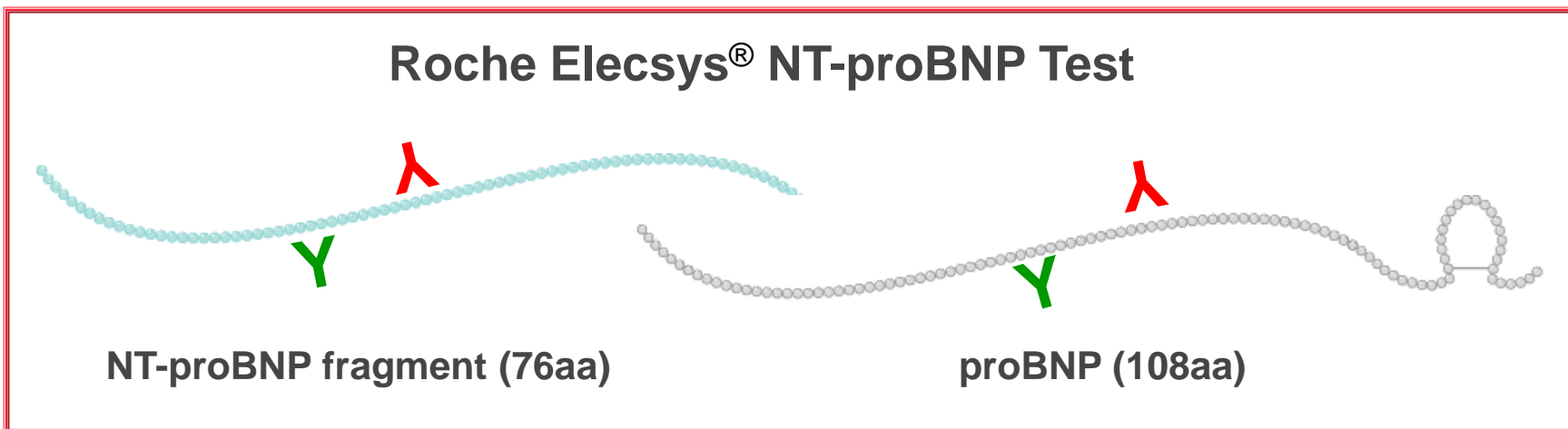
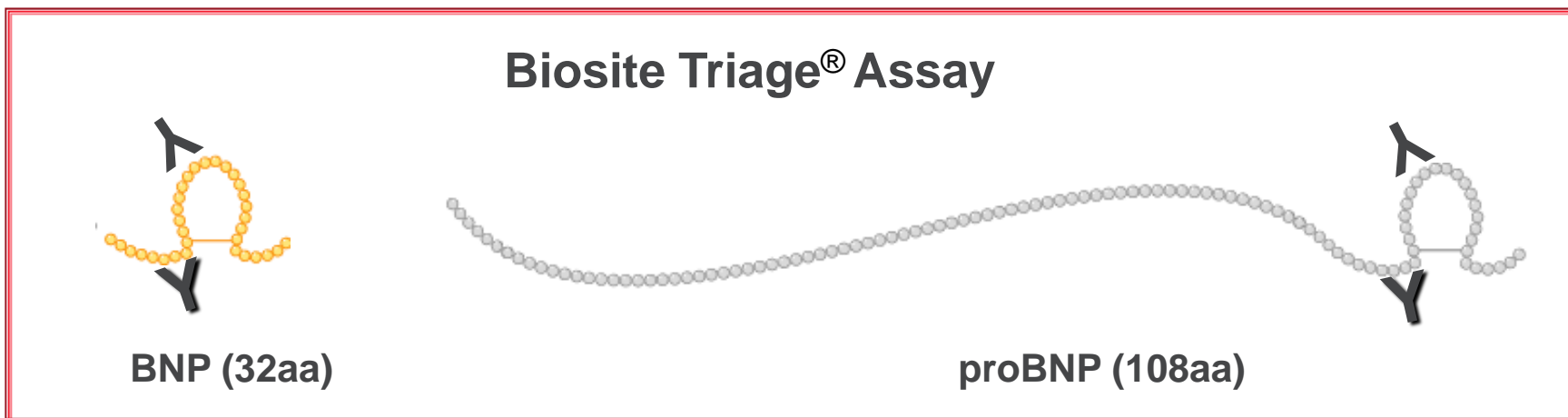
- AUC determines the clinical utility of biomarker with the described indication



Biomarker Application (Brain Natriuretic Peptide)

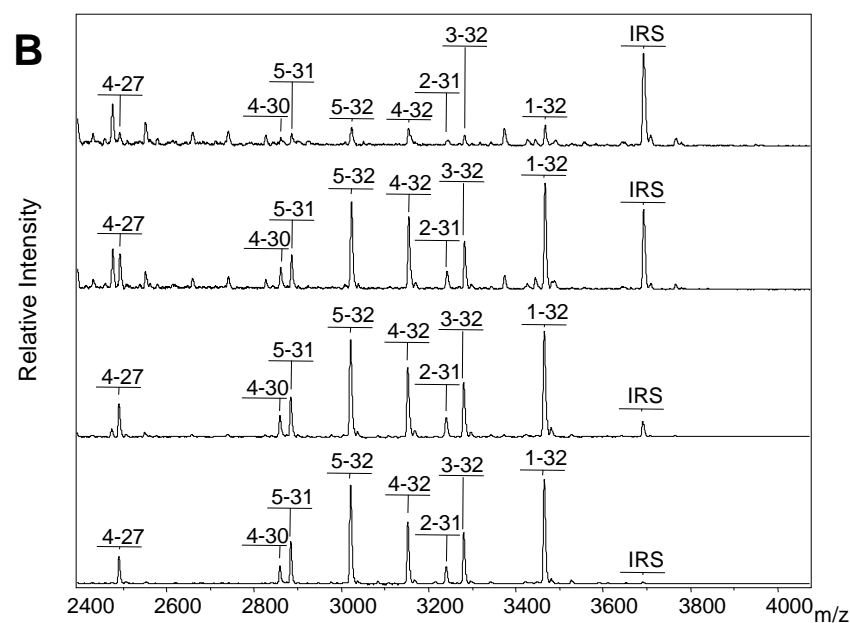
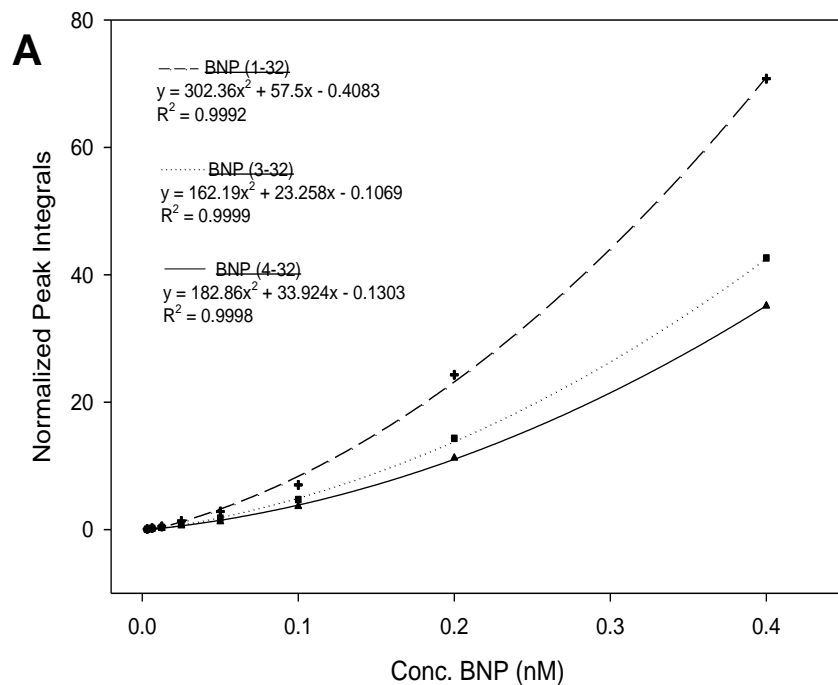
Application - MSIA™

Available immunoreactive assays cannot distinguish various circulating BNP forms



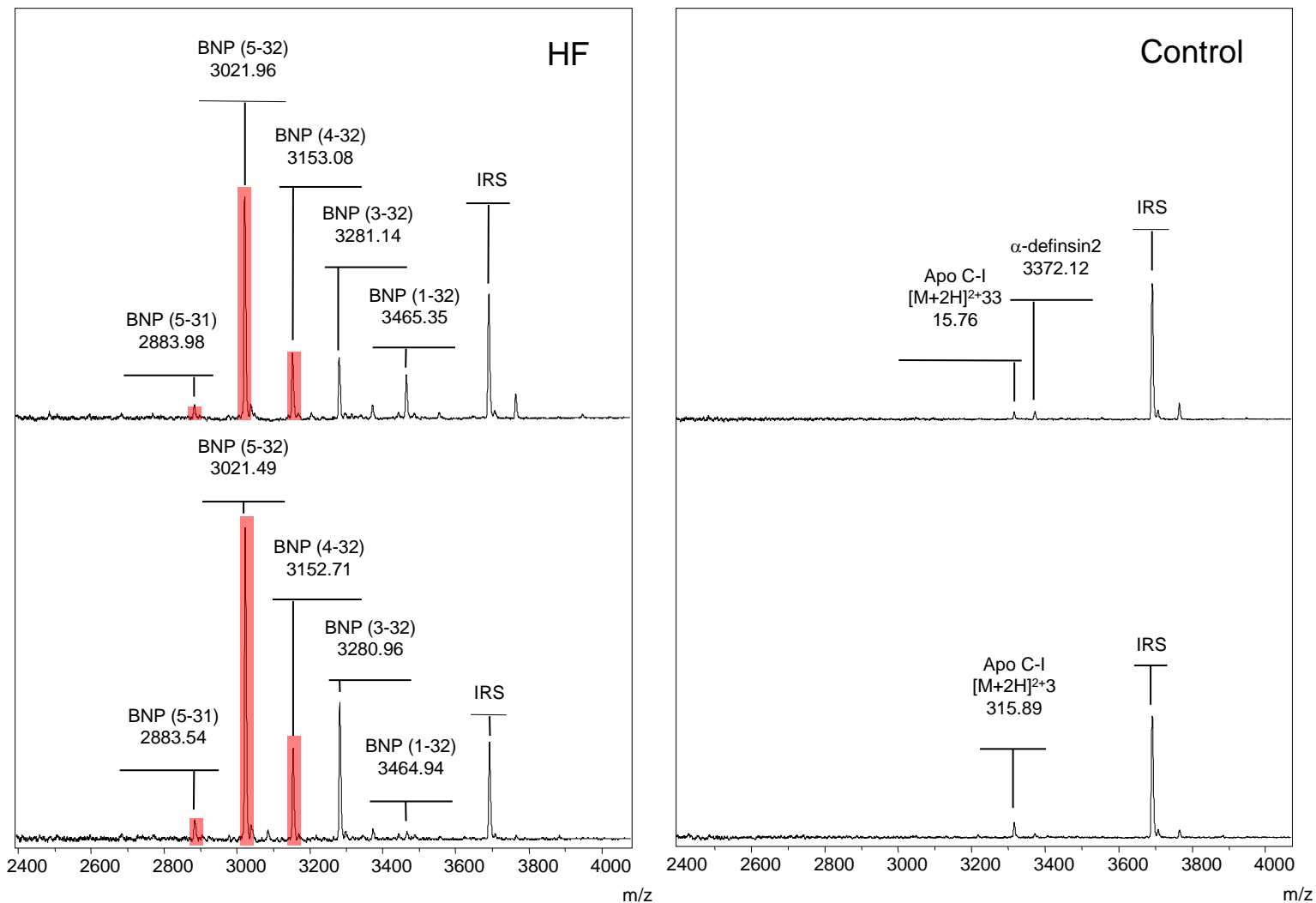
Application - MSIA™

BNP qMSIA™



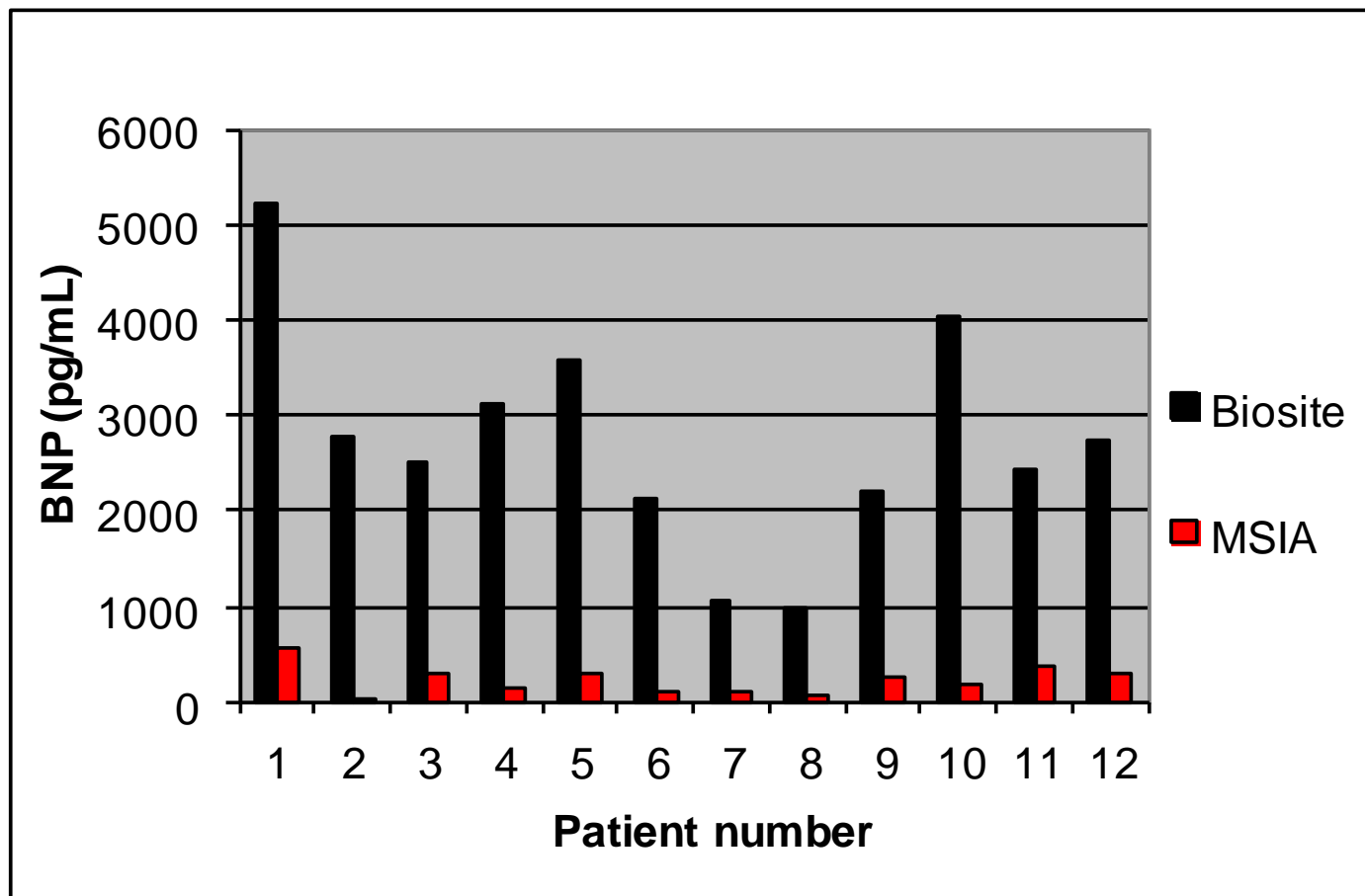
IRS: biotinylated BNP 1-32

Clinical Samples



Application – MSIA™ Comparative Data

qMSIA™ vs Biosite Triage assay



Circulation: Heart Failure, 1: 258-264, 2008

Biomarkers

- Confirmed the association between the protein variant (ApoCIII(1)) and the listed indications
- The ApoCIII(1) biomarker performed at level suggesting potential clinical utility
- Adds value to the hypothesis that specific isoforms of proteins have improved clinical utility

Technology

- Performed consistently and reproducibly
- Demonstrated a workflow that has utility in both discovery and validation applications
- Solidifies this approach as a viable method for the routine analysis of protein variation (**Population Proteomics**) *PNAS, 102: 10852-10857, 2005*

Summary

Technology

- Thermo Scientific MSIA Tips have the ability to rapidly capture and enrich proteins from human matrices for mass spectrometric detection
- Provide quantitative data of target analytes
- Use the MS x-axis to differentiate between protein subforms and extract potentially new clinical information

Application

- Provide data content that is lost in normal protein analytic methods.
- Demonstrated value in the use of examining protein micro-heterogeneity within disease states.