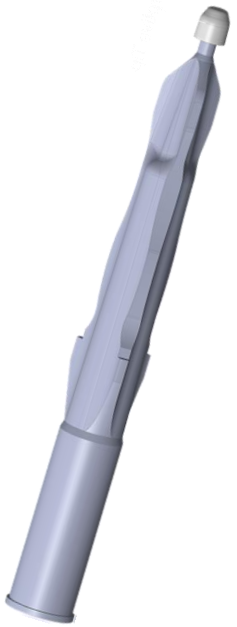
Cedars-Sinai Medical Center

Advanced Clinical Biosystems Research Institute
Bringing Discovery to Patient Care

Volumetric absorptive microsampling integrated into an **automated bottom-up proteomics** workflow

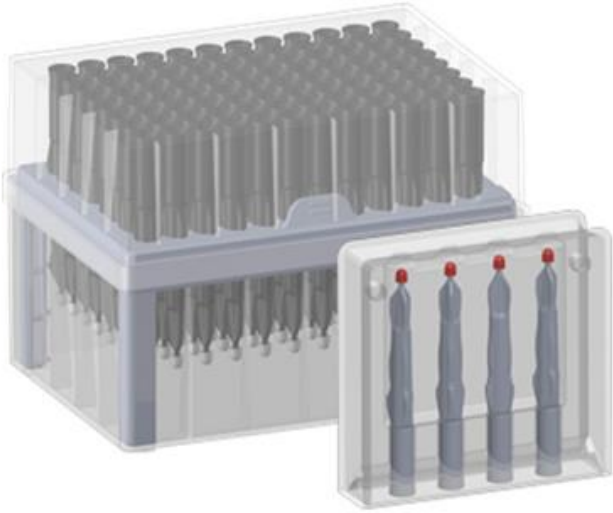


Irene van den Broek, PhD
Advanced Clinical Biosystems Research Institute,
Heart Institute, Cedars-Sinai Medical Center,
Los Angeles, CA

Mitra™ microsampler

Sampler Tip

- Fixed, highly reproducible internal volume, regardless of blood hematocrit level
- Dries <2hours
- Dried samples are considered non-biohazardous



Sampler Body

Ribs

- Prevent sample from contacting walls

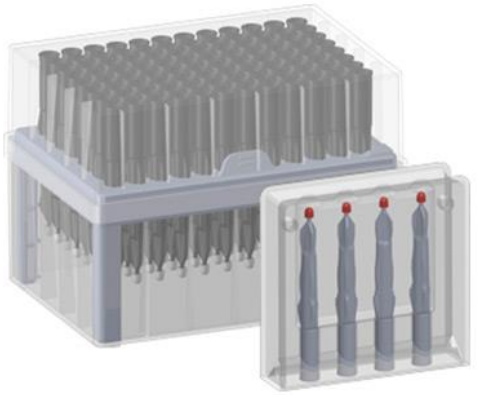
Barrel

- Can be written or labeled

Distal end

- Can fit most standard pipette heads

Mitra™ microsampler



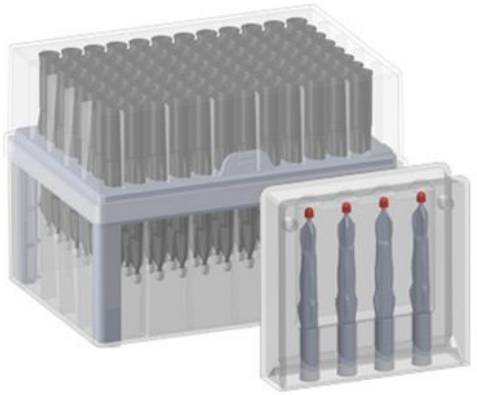
Finger prick
↓
Draw 10 µL blood
↓
Air dry
↓
Send to lab
(by mail)

- Less invasive
- No processing (e.g., centrifugation)
- No transfers / aliquots
- No freezing / thawing
- No courier transport (on ice)

Application to population-based proteomics studies?

- Applicable to proteins?
- Compatible with digestion?
- Amenable to automation?

Mitra™ microsampler



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Automated workflow

Extraction → Denaturation → Reduction → Alkylation → Digestion → Clean-up

Develop MRM assay →

Quantification

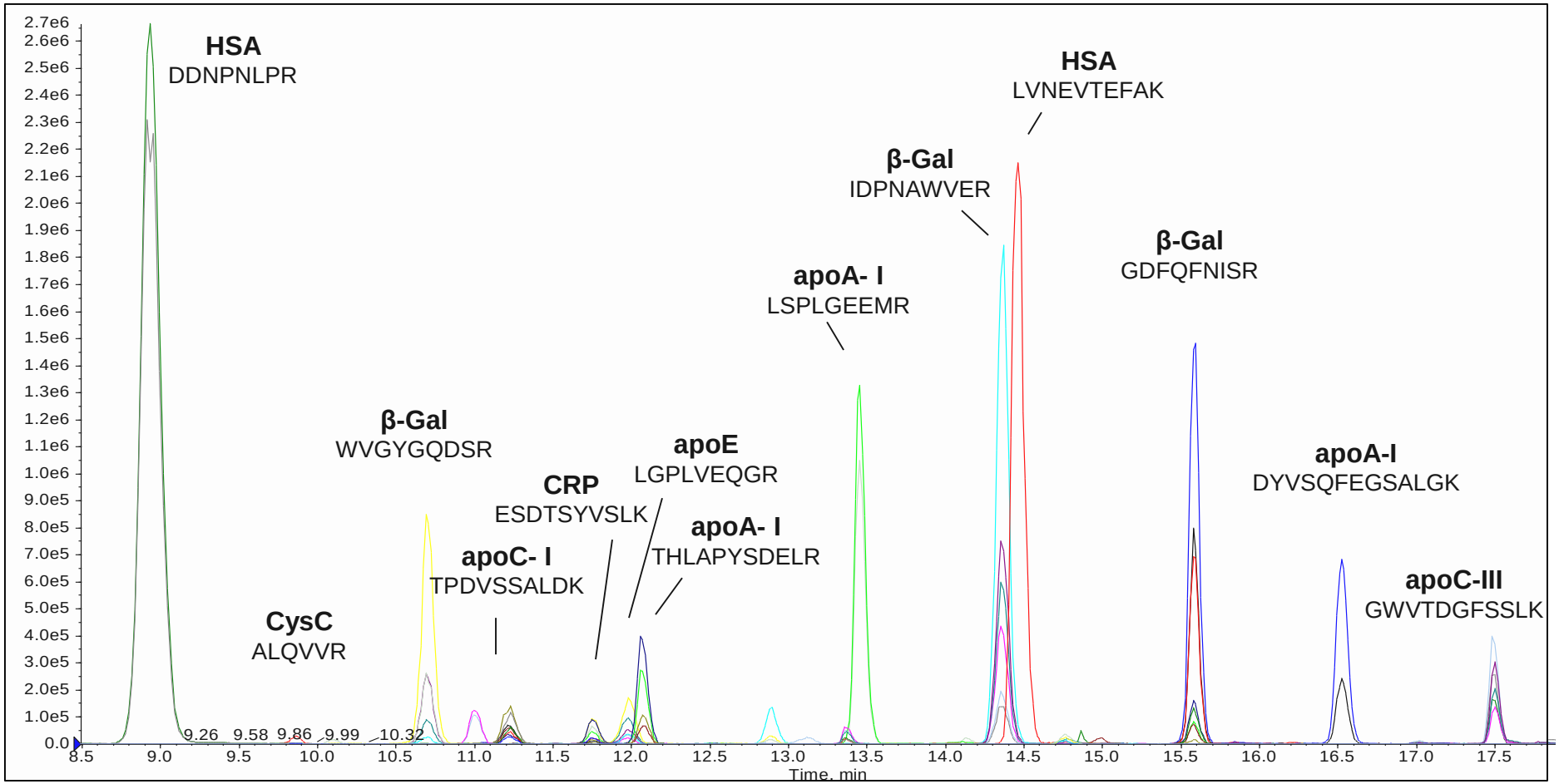
Develop MRM assay

Protein	Peptides	Role	Heavy peptide	LOD
β-Galactosidase	WVGYGQDSR	External control	✓	-
	IDPNAWVER	External control	✓	-
	GDFQFNISR	External control	✓	-
Human Serum Albumin	LVNEVTEFAK		✓	✓
	DDNPNLPR		✓	✓
Apolipoprotein A-I	THLAPYSDELR		✓	✓
	DYVSQFEGSALGK		✓	✓
	LSPLGEEM[ox]R/LSPLGEEMR	Oxidation monitoring	-	✓
	WQEEM[ox]ELYR/WQEEMELYR	Oxidation monitoring	-	✓
Apolipoprotein C-I	TPDVSSALDK		✓	✓
Apolipoprotein C-III	GWVTDGFSSLK		✓	✓
Apolipoprotein E	SELEEQLTPVAEETR		✓	✓
	AATVGSLAGQPLQER		✓	✓
	LGPLVEQGR		✓	✓
C-Reactive Protein	ESDTSYVSLK		✓	✗/✓
	GYSIFSYATK		✓	✗
Cystatin C	ALQVVR		✓	✓
	ALDFAVGEYNK		✓	✗
Periostin	GFEPGVTNILK		✓	✗
	IIDGVPVEITEK		✓	✗
	AAAITSDILEALGR		✓	✗

Develop MRM assay

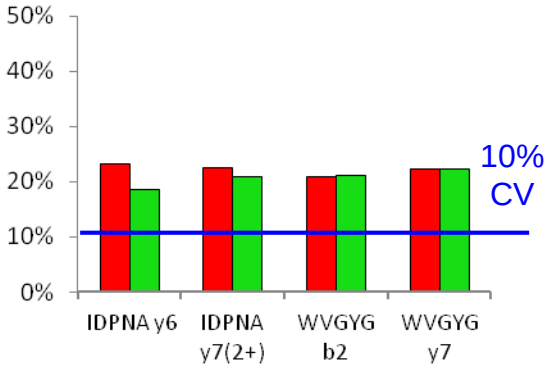
Prominence-20AD
(Shimadzu)

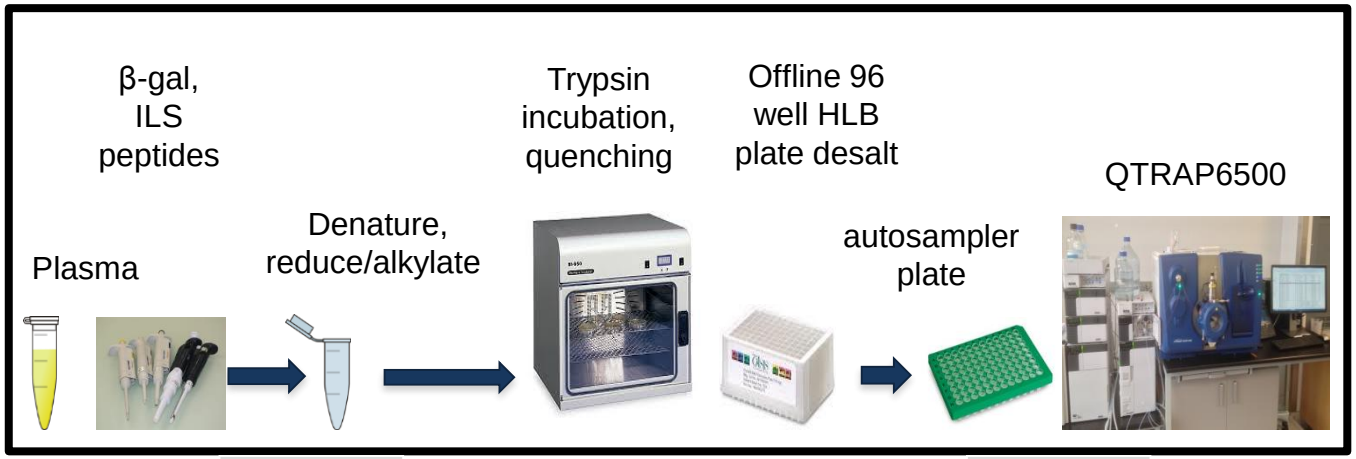
QTRAP® 6500
(Sciex)



NX^p Automation: Throughput and Robustness

Manual workflow





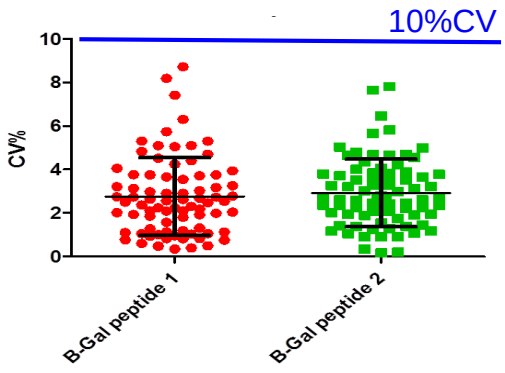
TIME

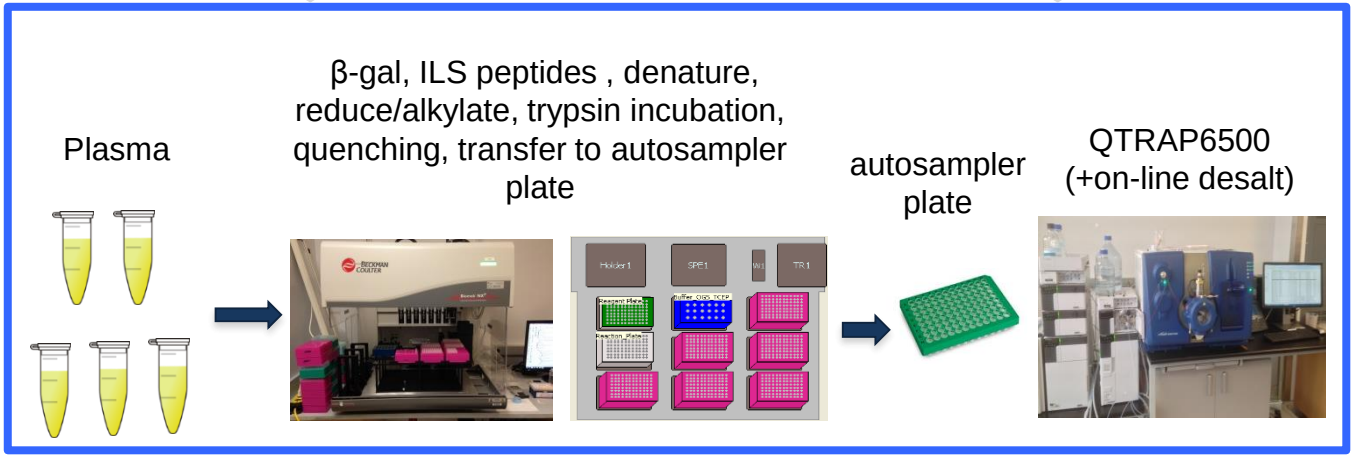
7 X reduction

CV%

> 4 X reduction

NX^p workflow

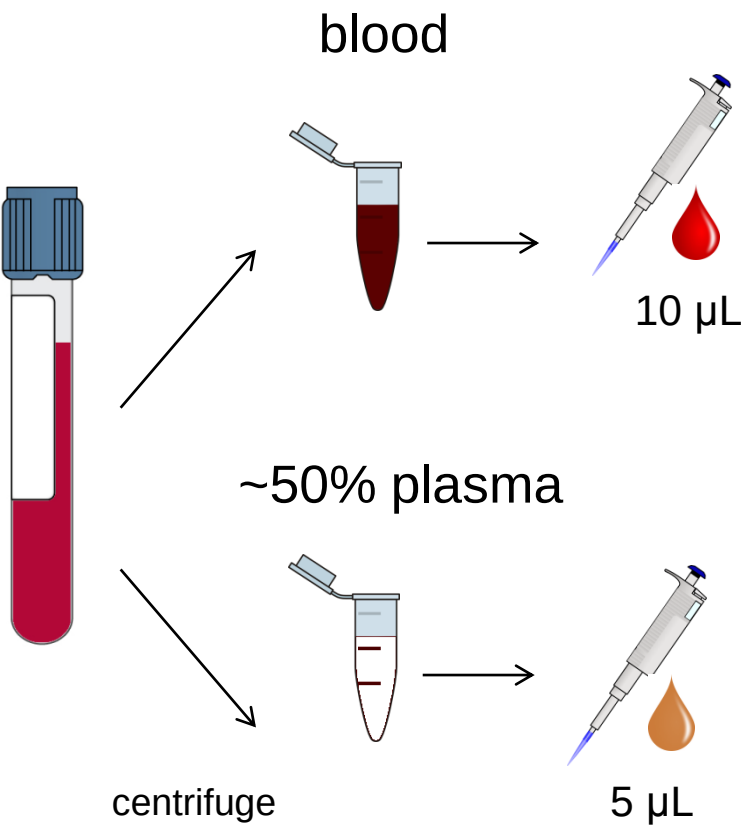




Qin Fu *et al*., Poster#13, Wednesday 5:00 PM

All Roads Lead to Robots: Automation of Customized, Effective Trypsin Digestion

Optimize Protein Digestion

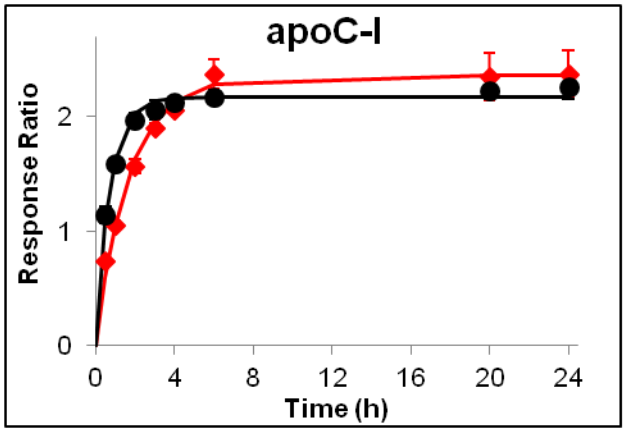


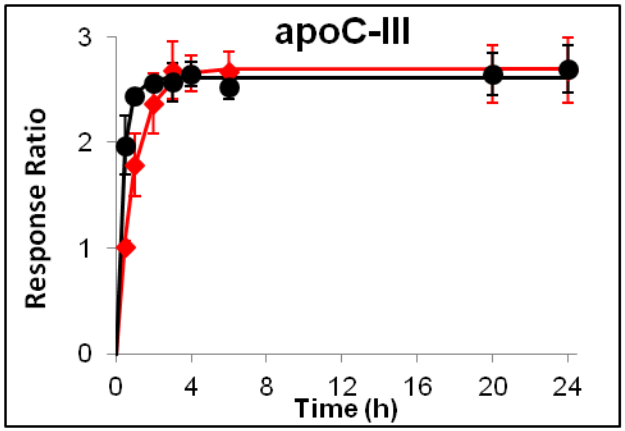
0.5h (n=3)	4h (n=3)
1h (n=3)	6h (n=3)
2h (n=3)	20h (n=3)
3h (n=3)	24h (n=3)

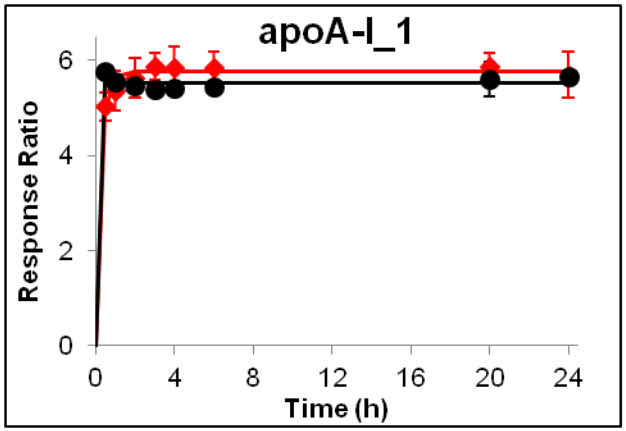
Denaturation / reduction / alkylation → Digestion → MRM

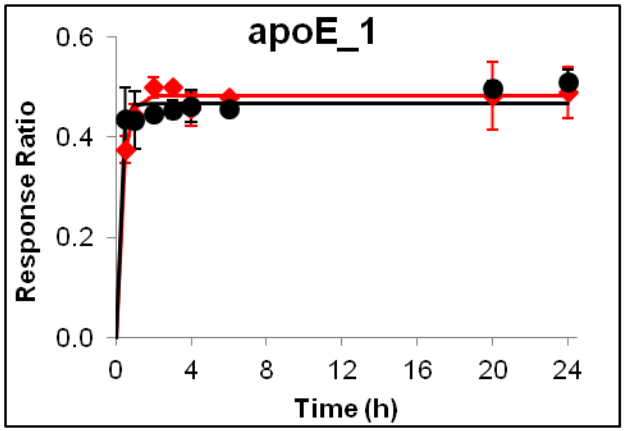
Can we adopt our plasma digestion protocol or does digestion protocol needs adjustment for application to (dried) blood samples?

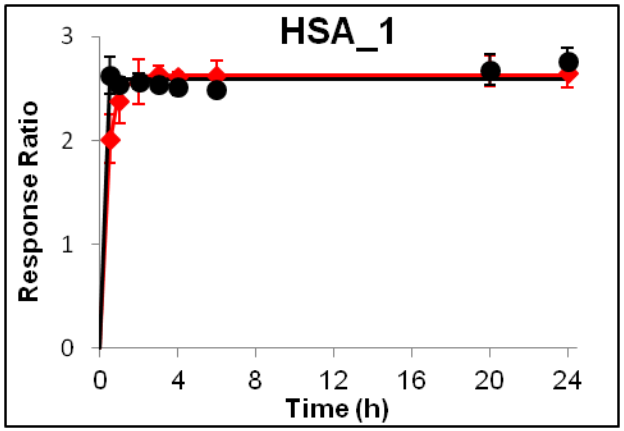
Optimize Protein Digestion



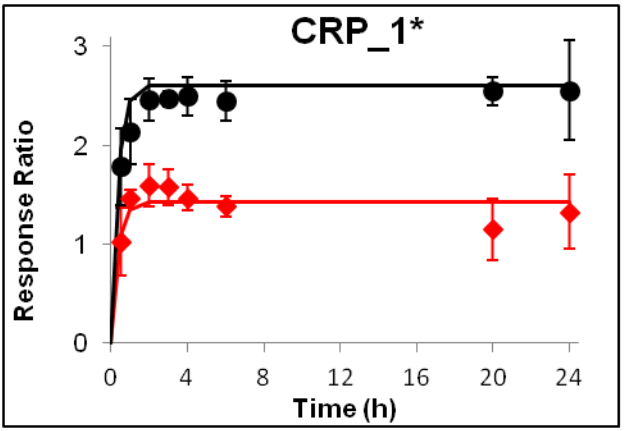


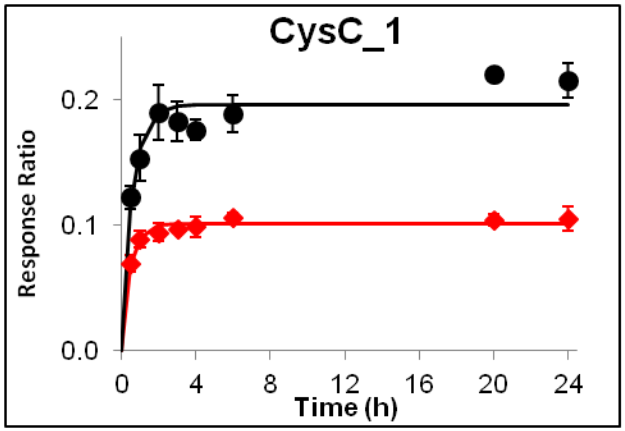




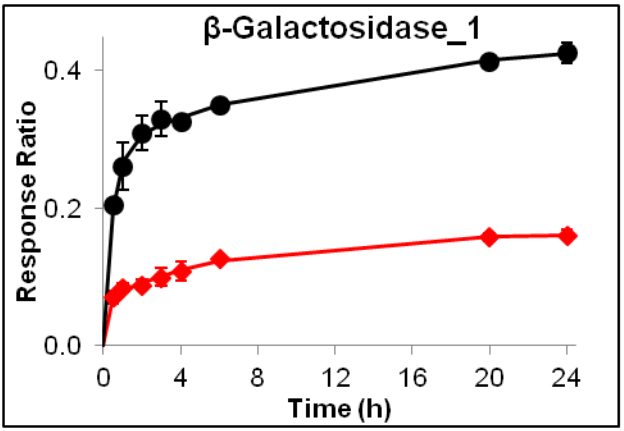


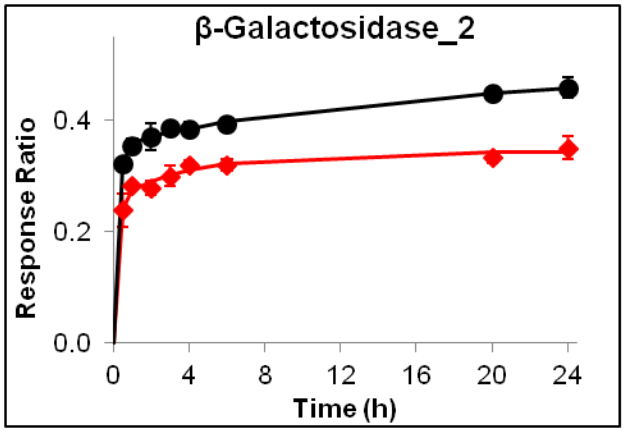
Optimize Protein Digestion

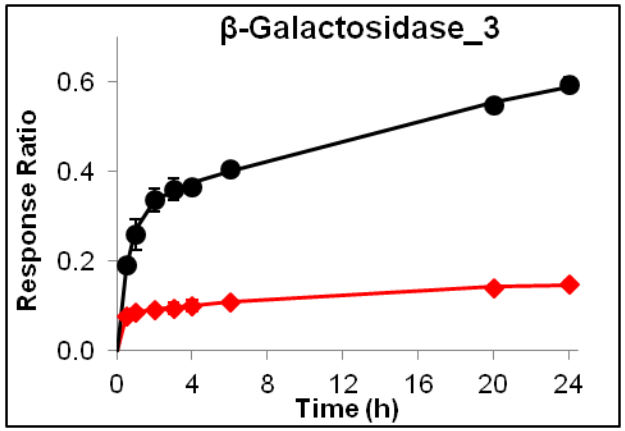




* rec. CRP spiked-in



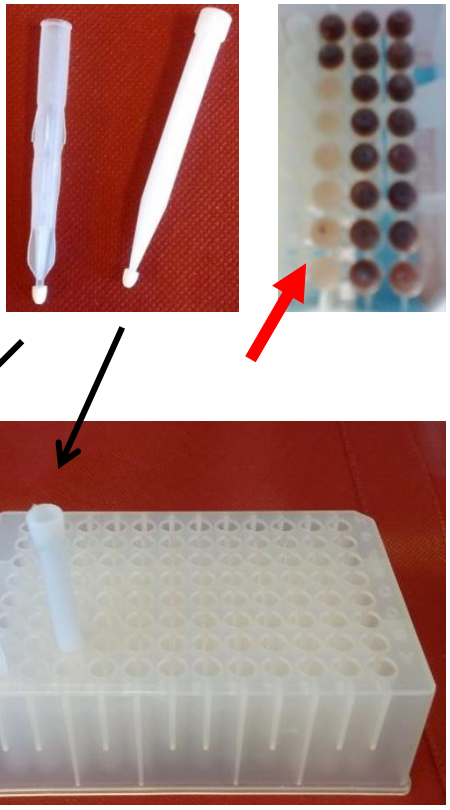


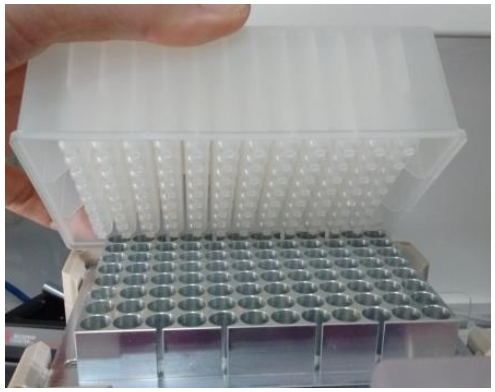


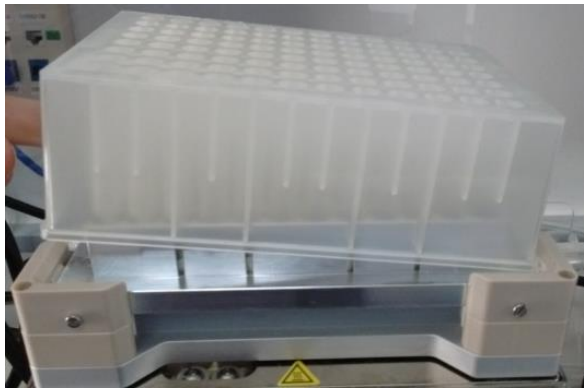
Optimize Protein Extraction

- **Compatibility with digestion:**
  - Digestion buffer (0.1 M Tris, 4 mM $CaCl_2$) works well for extraction
  - 150 µL extraction solvent better extraction efficiency than 100 µL
  - Addition of 20% (w/v) Octyl Glucoside (OGS) improved extraction efficiency

- **Compatibility with automation / 96-well format:**
  - Sonication limits standardization / automation
  - Sampler body does not fit into used 96 deep well plate

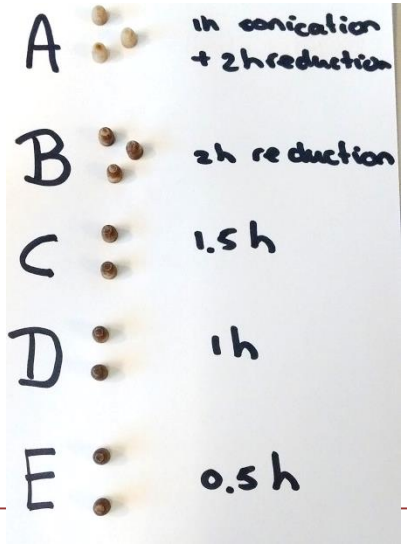


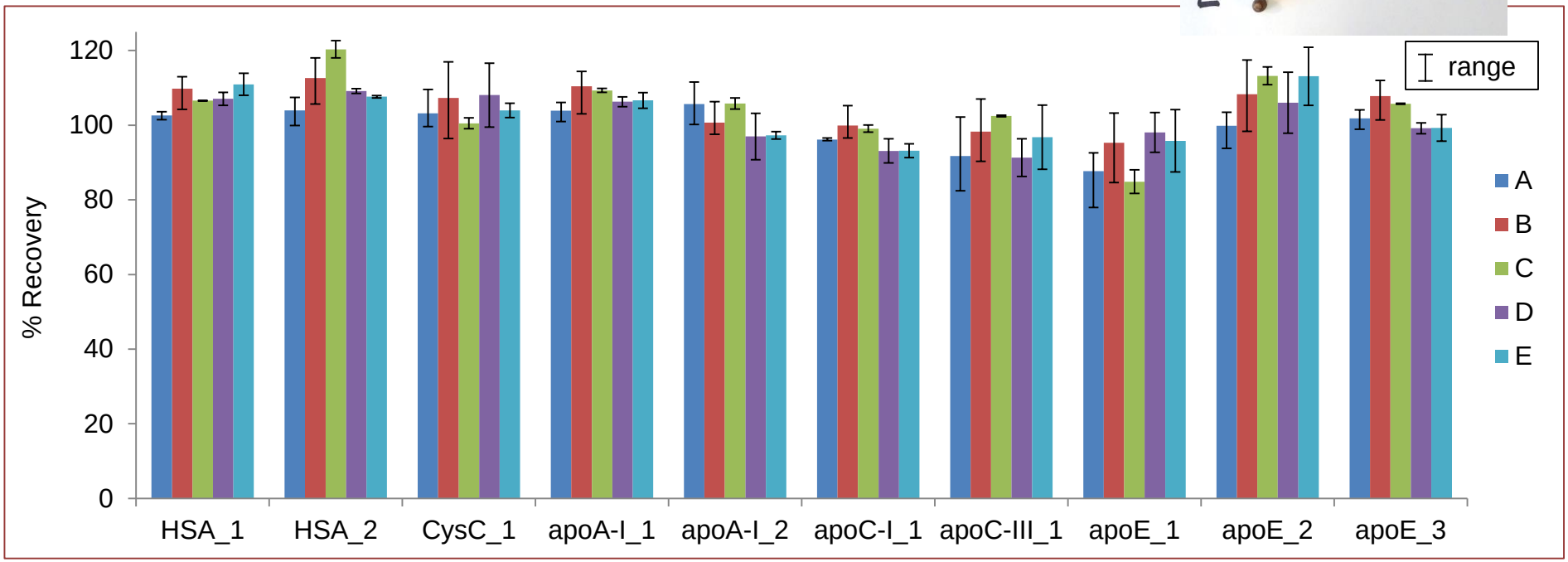




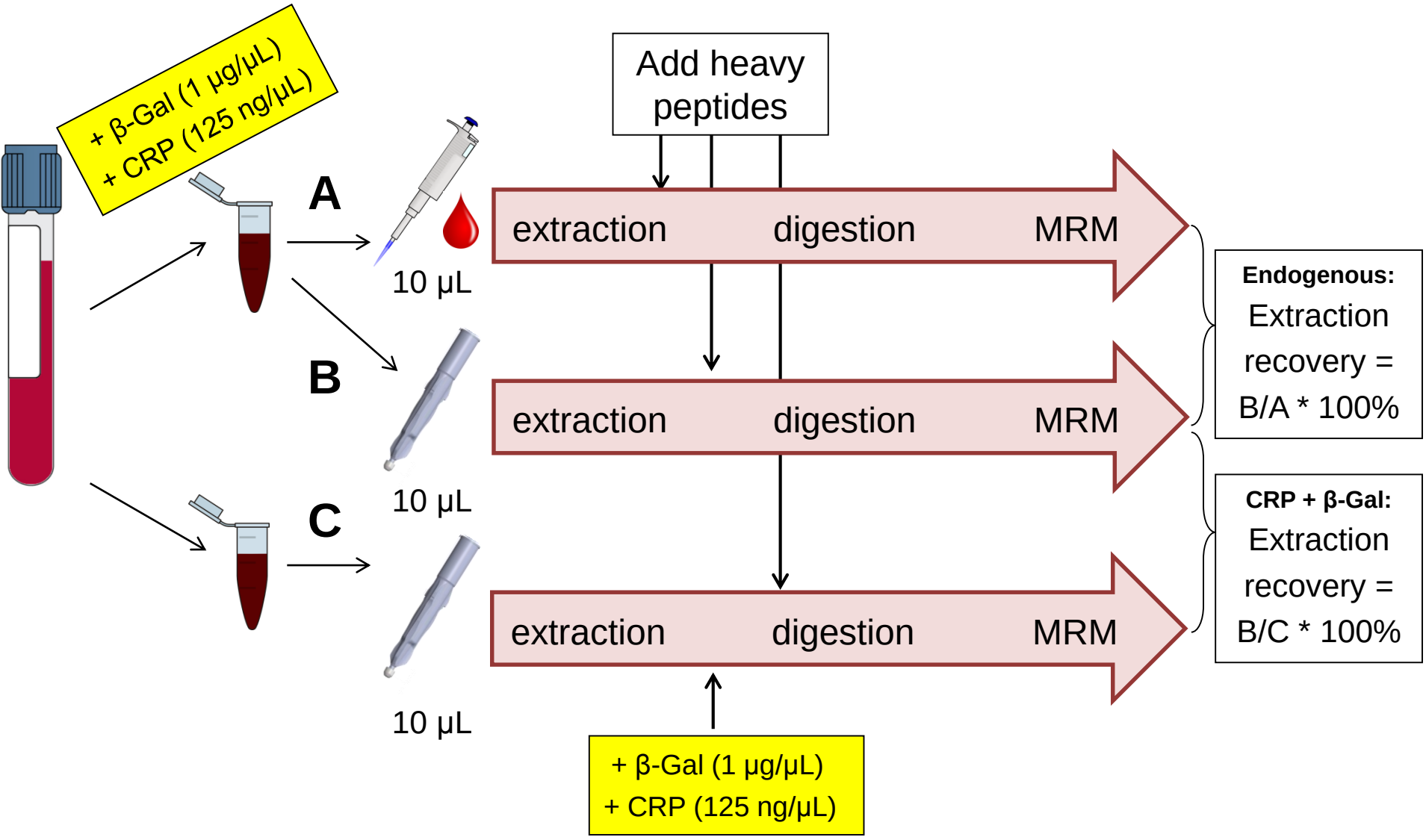
Combine Extraction and Digestion

A	n=3	1h sonication	2h reduction (60°C, 1200 RPM)
B	n=3	-	2h reduction (60°C, 1200 RPM)
C	n=2	-	1.5h reduction (60°C, 1200 RPM)
D	n=2	-	1.0h reduction (60°C, 1200 RPM)
E	n=2	-	0.5h reduction (60°C, 1200 RPM)





Extraction Recovery

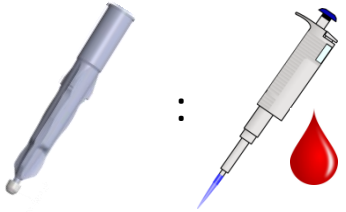


n=6 for each condition

Extraction Recovery (1)

Endogenous:

Extraction recovery = B/A * 100%

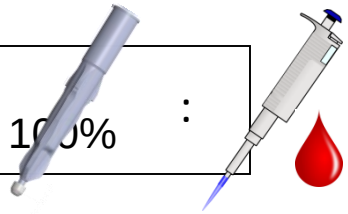


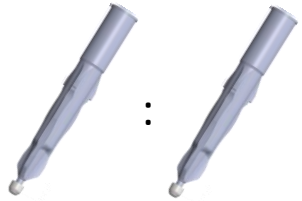
Protein	Peptide	Recovery ± CV (%) (n=6)
HSA	LVNEVTEFAK	103 ± 8%
HSA	DDNPNLPR	106 ± 7%
apoA-I	THLAPYSDELR	112 ± 8%
apoE	AATVGSLAGQPLQER	101 ± 6%
apoE	LGPLVEQGR	101 ± 5%
apoE	SELEEQLTPVAEETR	97 ± 5%
apoC-I	TPDVSSALDK	98 ± 8%
apoC-III	GWVTDGFSSLK	94 ± 6%
Cystatin C	ALQVVR	120 ± 9%

Extraction Recovery (2)

CRP + β-Gal:

Extraction recovery = B/C * 100%

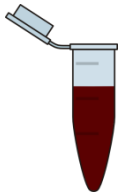




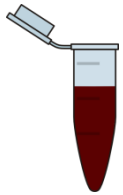
Pre-spike : Post-spike

Protein	Peptide	Recovery ± CV (%) (n=6)	
		B/A	B/C
β-Gal	GDFQFNISR	134 ± 8%	38 ± 7%
β-Gal	IDPNAWVER	114 ± 7%	109 ± 4%
β-Gal	WVGYGQDSR	118 ± 7%	46 ± 5%
CRP	ESDTSYVSLK	176 ± 23%	115 ± 11%

Reproducibility



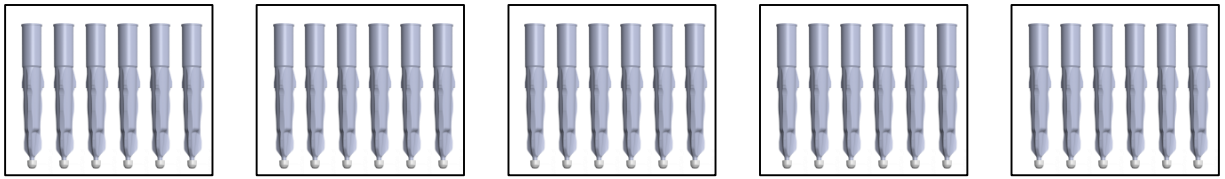
+ β-Gal (1 µg/µL)
+ CRP (125 ng/µL)



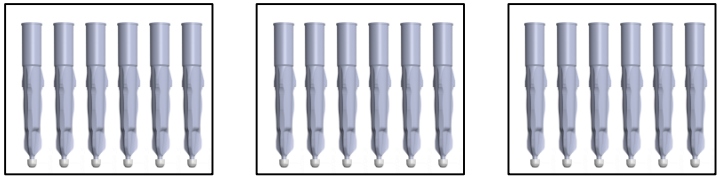
+ β-Gal (1 µg/µL)
+ CRP (125 ng/µL)

Sampled by 5 different persons
Work-up + analysis on same day

Operator 1 | Operator 2 | Operator 3 | Operator 4 | Operator 5



Day 1 | Day 2 | Day 3



Sampled by same person
Work-up + analysis on three days

Reproducibility (1)

			No normalization			β-Gal normalized		
Protein	**Peptide**	**LC-MS/MS %CV (n=6)**	**Intra-operator %CV**	**Inter-operator %CV**	**Total %CV**	**Intra-operator %CV**	**Inter-operator %CV**	**Total %CV**
β-Gal	GDFQFNISR	1.8	5.8	10.0	11.5	-	-	-
β-Gal	IDPNAWVER	1.2	4.7	9.1	10.3	-	-	-
β-Gal	WVGYGQDSR	1.1	5.2	9.9	11.2	-	-	-
HSA	LVNEVTEFAK	1.9	4.8	8.9	10.1	2.3	0.9	2.5
apoA-I	THLAPYSDELR	2.5	4.4	8.2	9.3	3.4	0.7	3.5
apoE	AATVGSLAGQPLQER	3.0	4.2	8.0	9.0	2.4	1.8	3.0
apoE	LGPLVEQGR	3.0	5.7	8.4	10.2	3.0	3.9	3.9
apoE	SELEEQLTPVAEETR	6.0	6.5	6.9	9.5	5.2	3.3	6.2
apoC-I	TPDVSSALDK	3.0	4.9	7.8	9.2	3.2	1.8	3.7
apoC-III	GWVTDGFSSLK	2.8	4.7	6.2	7.8	4.2	2.5	4.8
CRP	ESDTSYVSLK	15.0	9.3	6.1	11.1	9.0	3.8	9.8
Cystatin C	ALQVVR	6.5	9.2	11.5	14.7	7.2	3.1	7.8

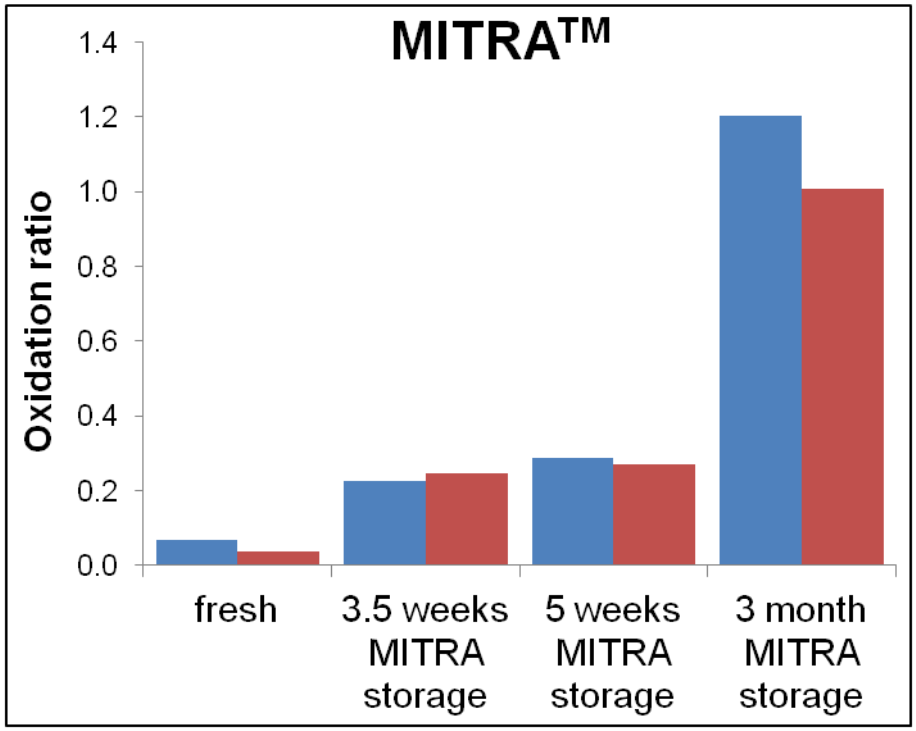
Evaluation of inter-operator variability: Denniff and Spooner, Anal Chem **2014**

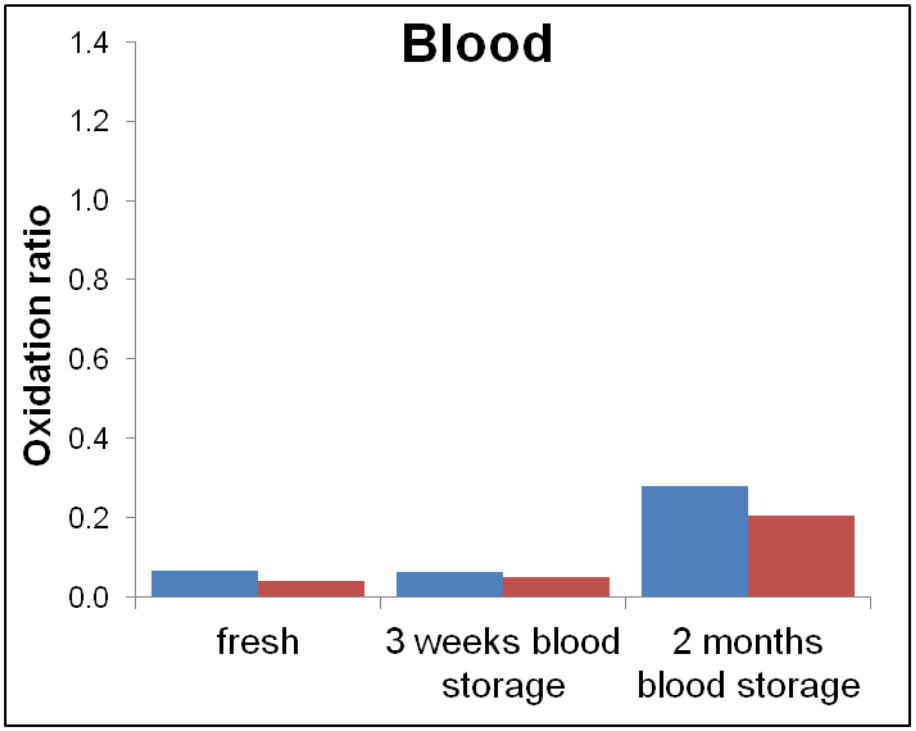
Reproducibility (2)

Protein	Peptide	Intraday %CV (n=6; d=3)	Total %CV
β-Gal	GDFQFNISR	9.7	11.0
β-Gal	IDPNAWVER	8.1	8.1
β-Gal	WVGYGQDSR	7.7	8.7
HSA	LVNEVTEFAK	5.4	16.3
HSA	DDNPNLPR	6.6	13.7
apoA-I	THLAPYSDELR	7.7	>>
apoE	AATVGSLAGQPLQER	8.0	8.0
apoE	LGPLVEQGR	7.3	7.3
apoE	SELEEQLTPVAEETR	7.5	8.2
apoC-I	TPDVSSALDK	7.8	>>
apoC-III	GWVTDGFSSLK	8.9	>>
CRP	ESDTSYVSLK	10.0	10.0
Cystatin C	ALQVVR	8.0	8.0

apoA-I oxidation

LSPLGEEM[ox]R/LSPLGEEMR WQEEM[ox]ELYR/WQEEMELYR





- Don’t use methionine for quantification, but:
- Monitoring of methionine oxidation might be used as an indicator for quality of sample storage / handling / processing

Conclusions / Follow-up

- The extraction of proteins from MITRA™ tips can be integrated with a typical bottom-up proteomics workflow

- With automated procedure for protein extraction and digestion:
  - Proteins were efficiently extracted from MITRA™ tips (~100% recovery)
  - Protein digestion was comparable to our optimized protocol for plasma
  - Proteins were reproducibly measured (<5-10 %CV)

- Ongoing studies focus on:
  - Evaluation of **protein stability** in Mitra tips under various conditions
  - **Optimization of digestion** as compared to plasma
  - **Optimization of sample clean-up** for lower abundance proteins
  - Selection of analyte(s) for **application to clinical samples**

Acknowledgments

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Mitra Mastali

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Jennifer van Eyk

Department of Surgery, Division of Urology:

Lenny Ackerman

neoteryx™
The promise of microsampling, delivered

Stuart Kushon

Kim Chansky

Bobby Virasingh

Cambridge Isotope Laboratories

Kevin Millis

Krista Backiel

Tasha Agreste

BECKMAN COULTER
Life Sciences

Mike Kowalski

Alice Tanibata-Branham

Tara Jones-Roe