C-Peptide Standardization

JCTLM Members' and Stakeholders' Meeting

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BIPM, Sevres

Randie R. Little, Ph.D.

Diabetes Diagnostic Laboratory (DDL), University of Missouri School of Medicine, Columbia, MO

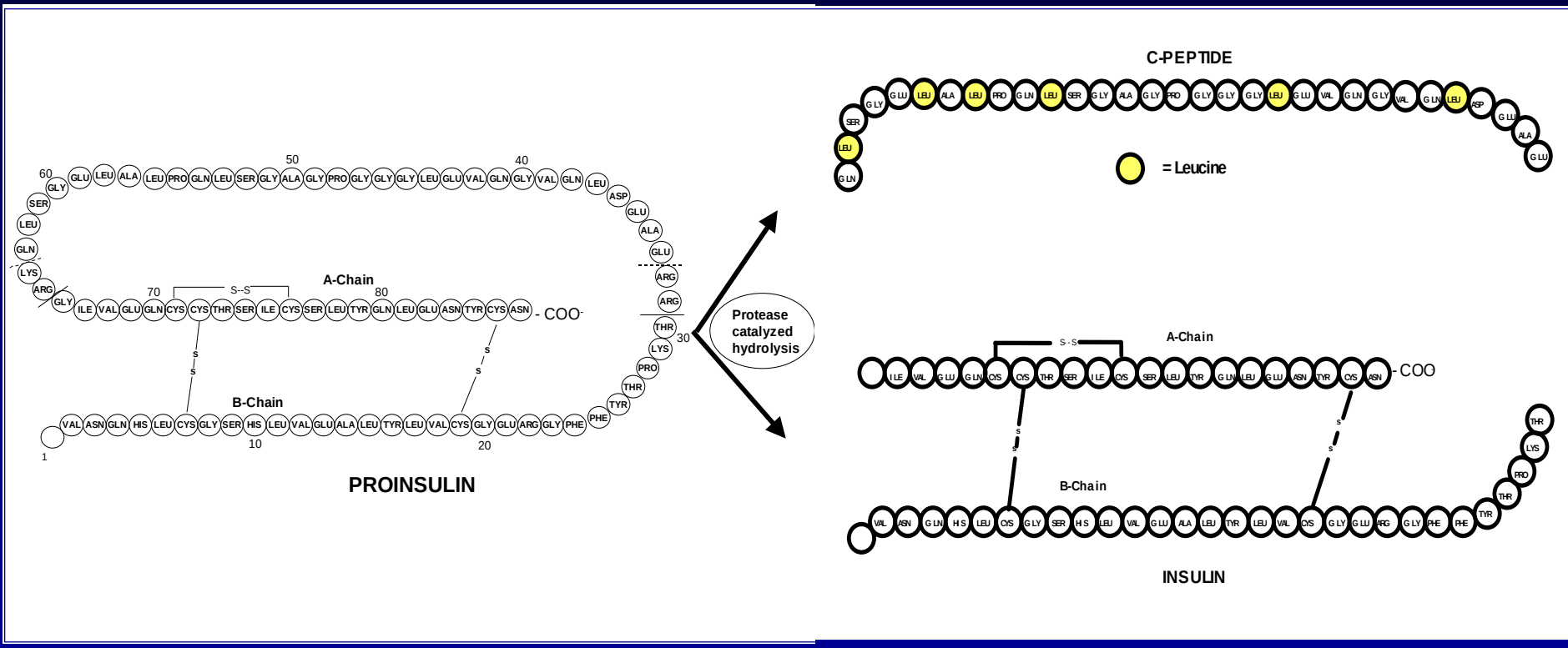
C-Peptide Standardization

- Clinical need
- C-peptide Standardization efforts
- Current issues with calibration/Reference Materials
- Next steps

Clinical Need

- > 1.4 million people with Type 1 diabetes in the US and the incidence rates rising
- Type 1 diabetes is an autoimmune disease.
- Preventing future, maintaining and/or restoring beta cell function is the goal.
- C-peptide is the most accurate biomarker of beta cell function in beta cell-depleted diabetes.

Pro-insulin is cleaved into Insulin and C-peptide



C-peptide is a marker of Beta cell function

- Pro-insulin is synthesized in the pancreatic beta cells
- Pro-insulin is packaged into granules and cleaved to insulin and C-peptide.
- Insulin and C-peptide are secreted in a 1:1 molar ratio.
- Insulin (but not C-peptide) is cleared by the liver
- C-peptide is the best marker of insulin secretion

Why preserve Beta cell function?

Among DCCT subjects in the intensive treatment group:

- Prevented short term complications, e.g. hypoglycemia
- Prevented long-term complications, e.g. retinopathy, neuropathy, nephropathy, heart attack, stroke

C-Peptide Standardization Efforts

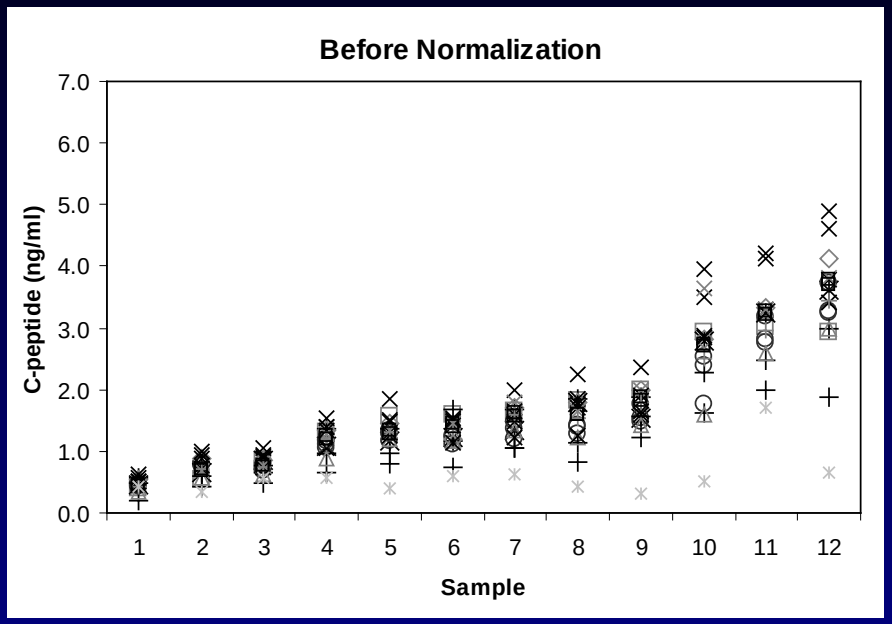
Background

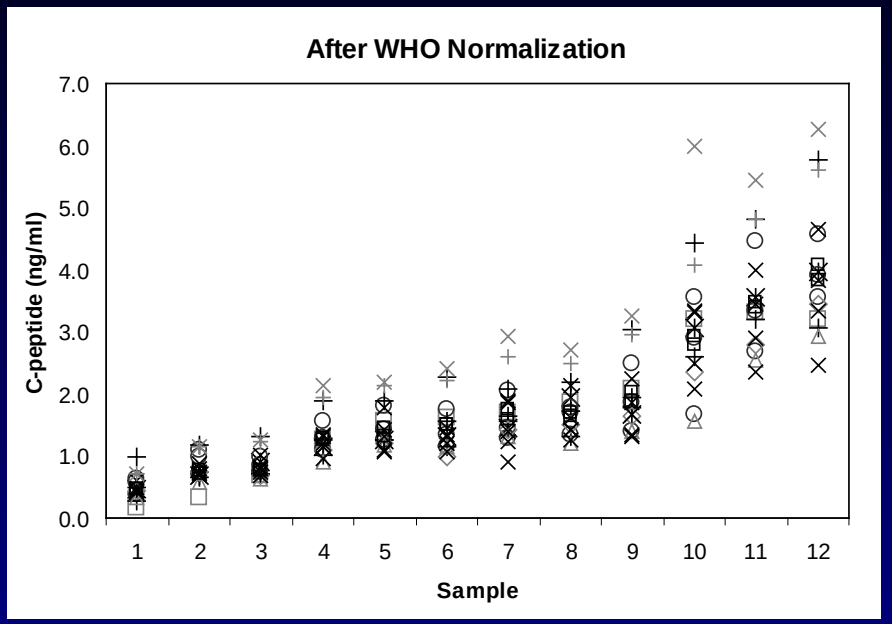
In 2002, the NIDDK organized a C-peptide standardization committee and funded an international comparison study of C-peptide assays.

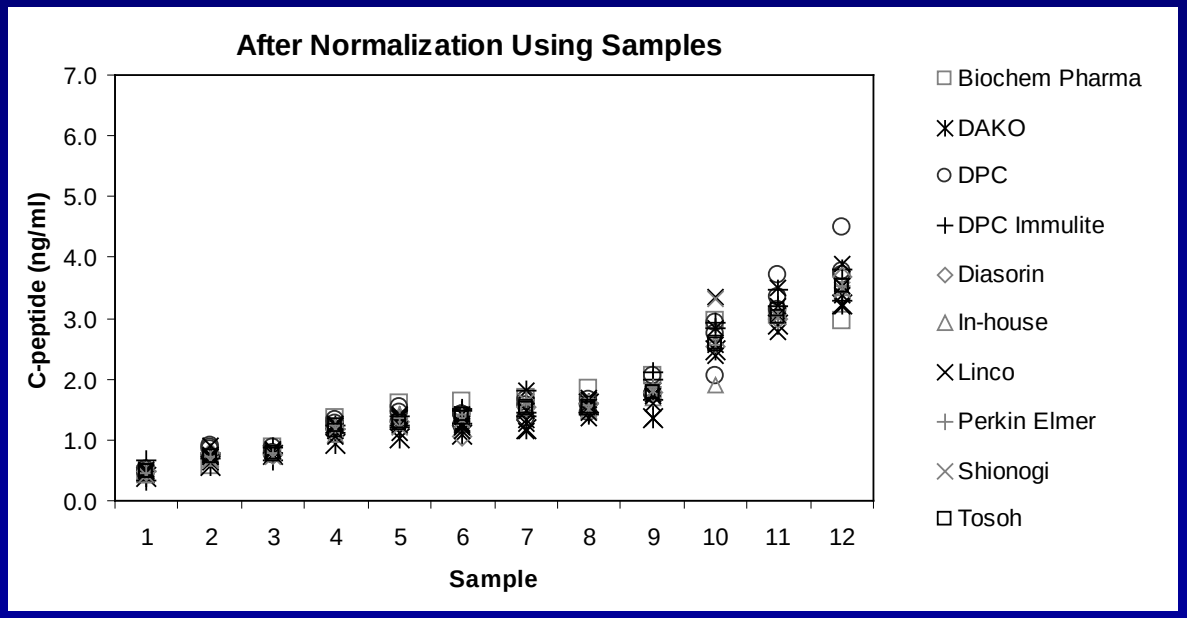
Phase I Studies: Purpose

The goal of the initial studies was to assess the degree of comparability of C-peptide results and to determine whether C-peptide results could be normalized.

Phase I: Study Results







Phase I: Results

- After normalization with **WHO standard**, the 95% CI estimate for the SD for the lab/method effect overlapped with the 95% CI estimated with the raw data.
- After normalization with **samples**, the 95% CI estimate for the SD for the lab/method effect did not overlap with the 95% CI estimated with the raw data.

Phase I: Conclusions

- WHO normalization was ineffective in reducing the variability of C-peptide results within and among lab/methods.
- Normalization with sample calibrators was effective in reducing variability of C-peptide results.

Phase II Studies: Purpose

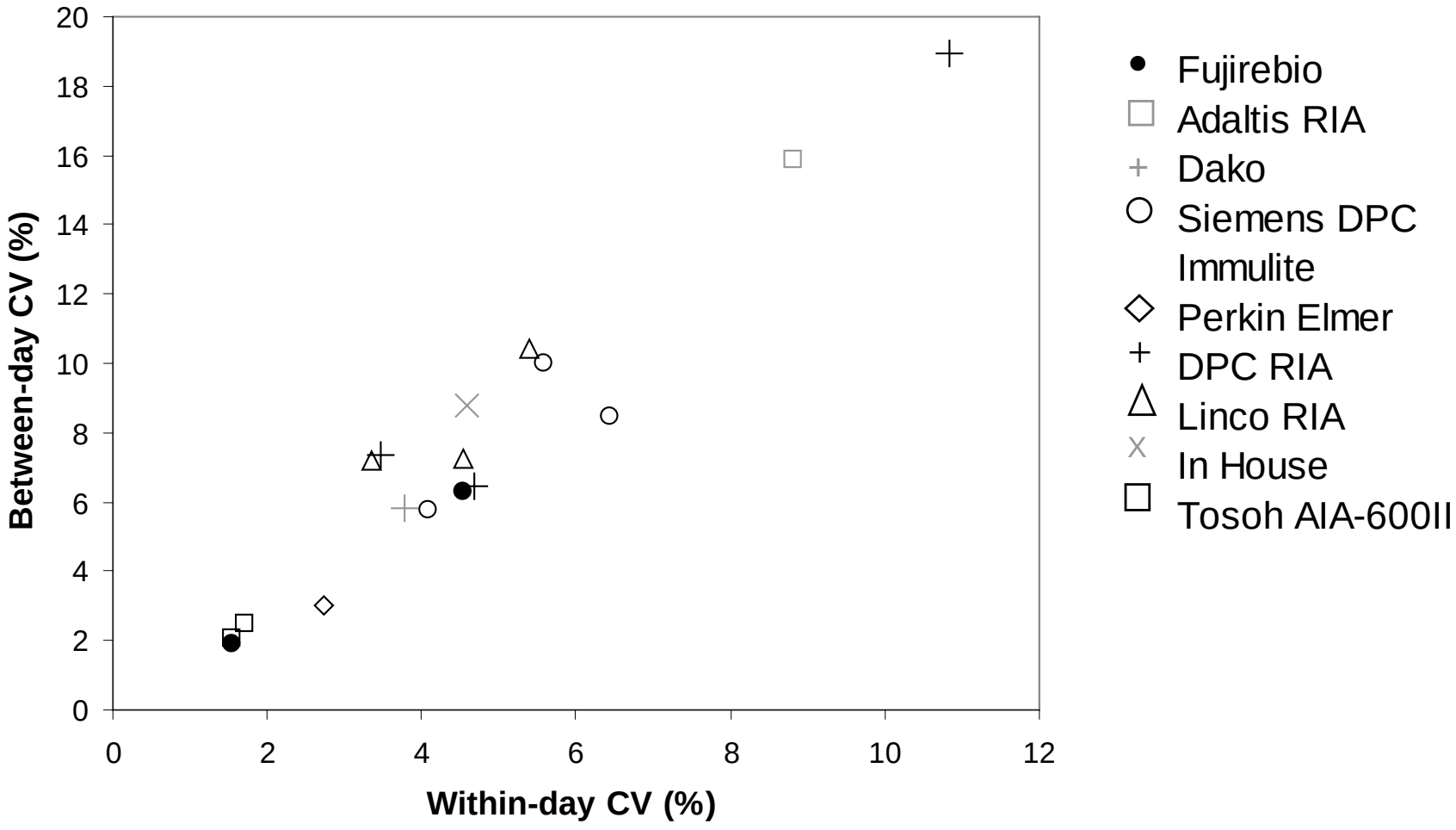
The goal of the 2nd phase studies was to

- evaluate the use of a Reference Method (Stein, el al) for assigning values to sample matrix calibrators.
- estimate precision of each method

Phase II: Methods

- Forty different serum samples (fasting and post-prandial) from non-diabetic subjects shipped on dry ice to 15 laboratories using 9 different methods.
- Matched EDTA plasma samples (with added Aprotinin) were also sent to Stein, et al (Bronx, NY) for analysis with a proposed reference method (LC-MS).

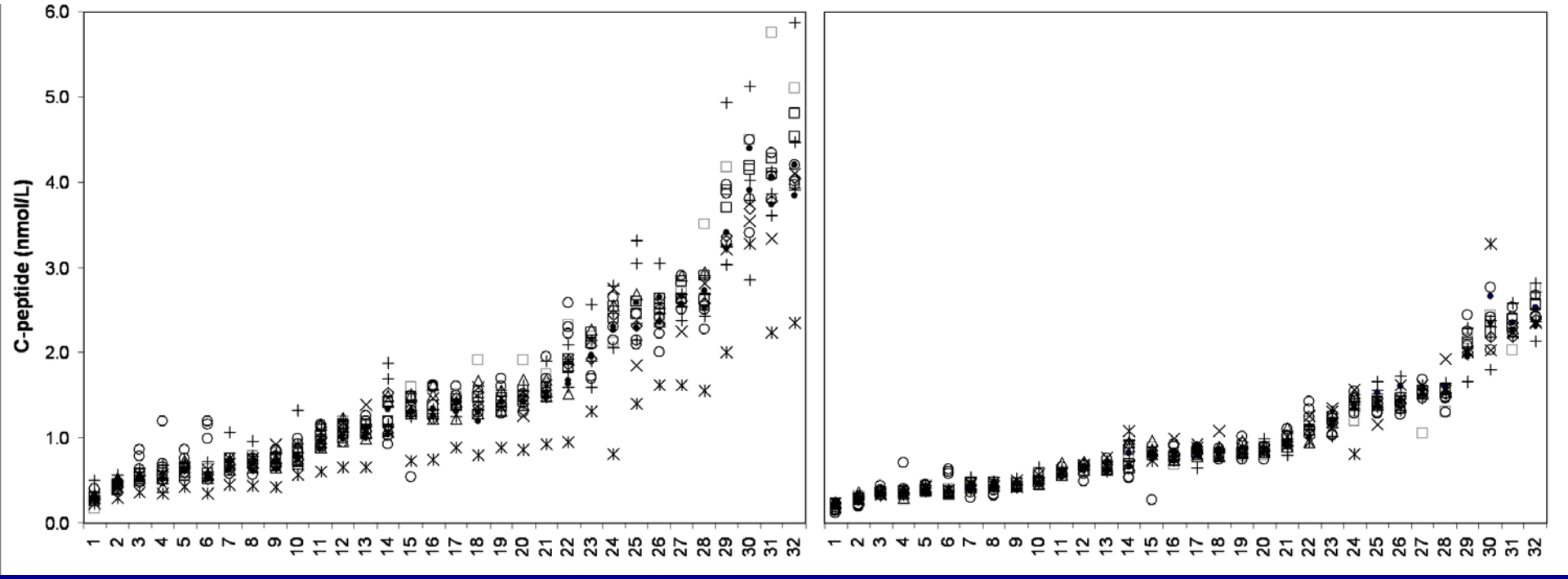
Phase II: Results



Phase II: Results

Before Normalization

After Normalization to a Ref Method



Phase II: Results

- Before normalization there were significant differences between laboratory means ($p<0.0001$). The least-squares means ranged from 1.55 – 1.95.
- After normalization there were no significant differences in the mean responses ($p=0.24$). The least-squares means ranged only from 0.93 to 1.02.

Phase II: Conclusions

Normalization of C-peptide results using patient samples that have been assigned values by a reference method greatly reduces the variability among methods and laboratories.

Phase III Studies: Purpose

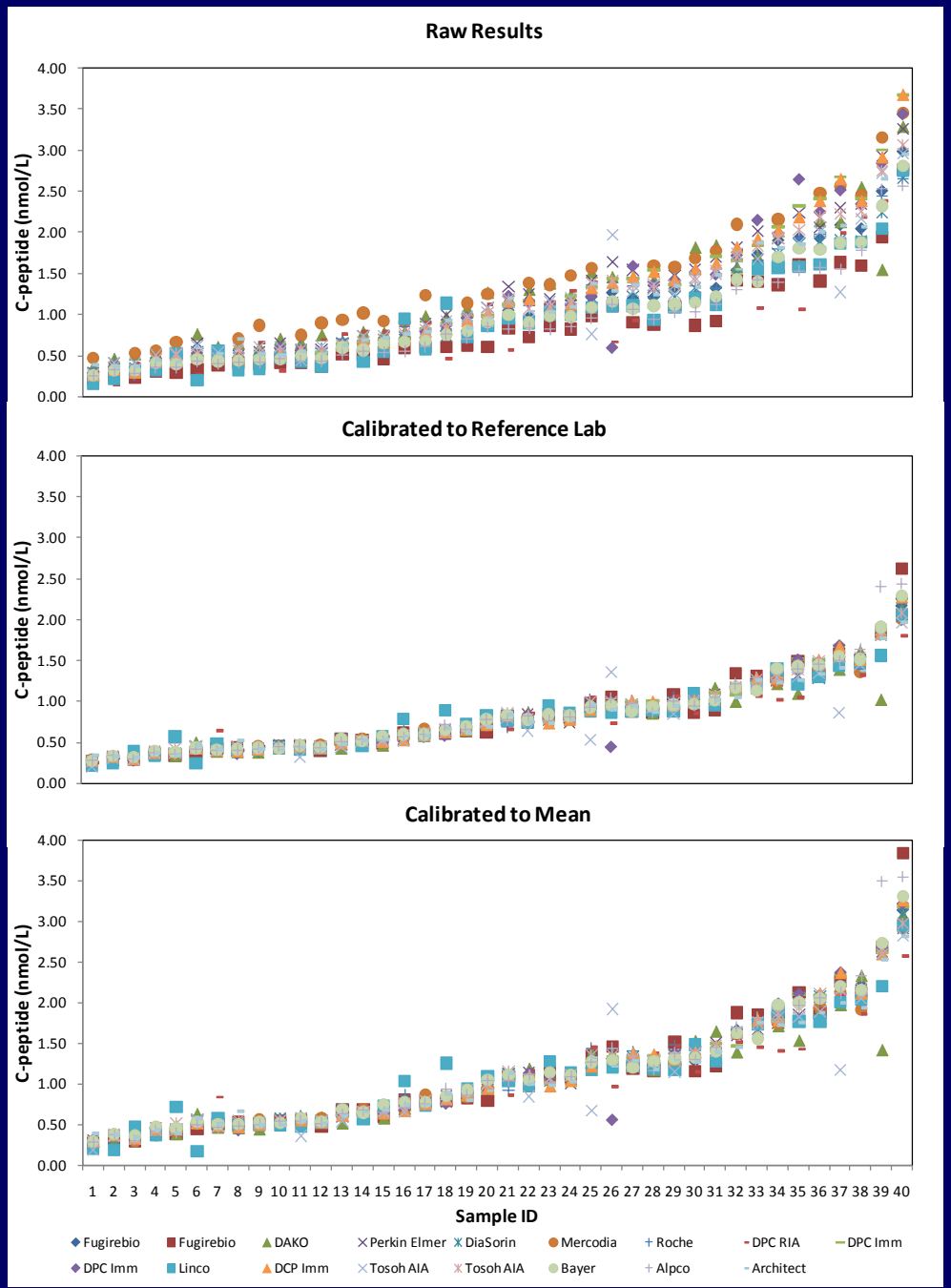
The goal of the 3rd phase studies was to

- Verify the use of the Ref Method to assign calibrator values
- compare the use of single-donor samples for calibrators to pooled samples
- Include additional methods
- evaluate different matrices for Reference Method (serum, EDTA+Aprotinin, serum+Aprotinin)
- estimate precision of each method

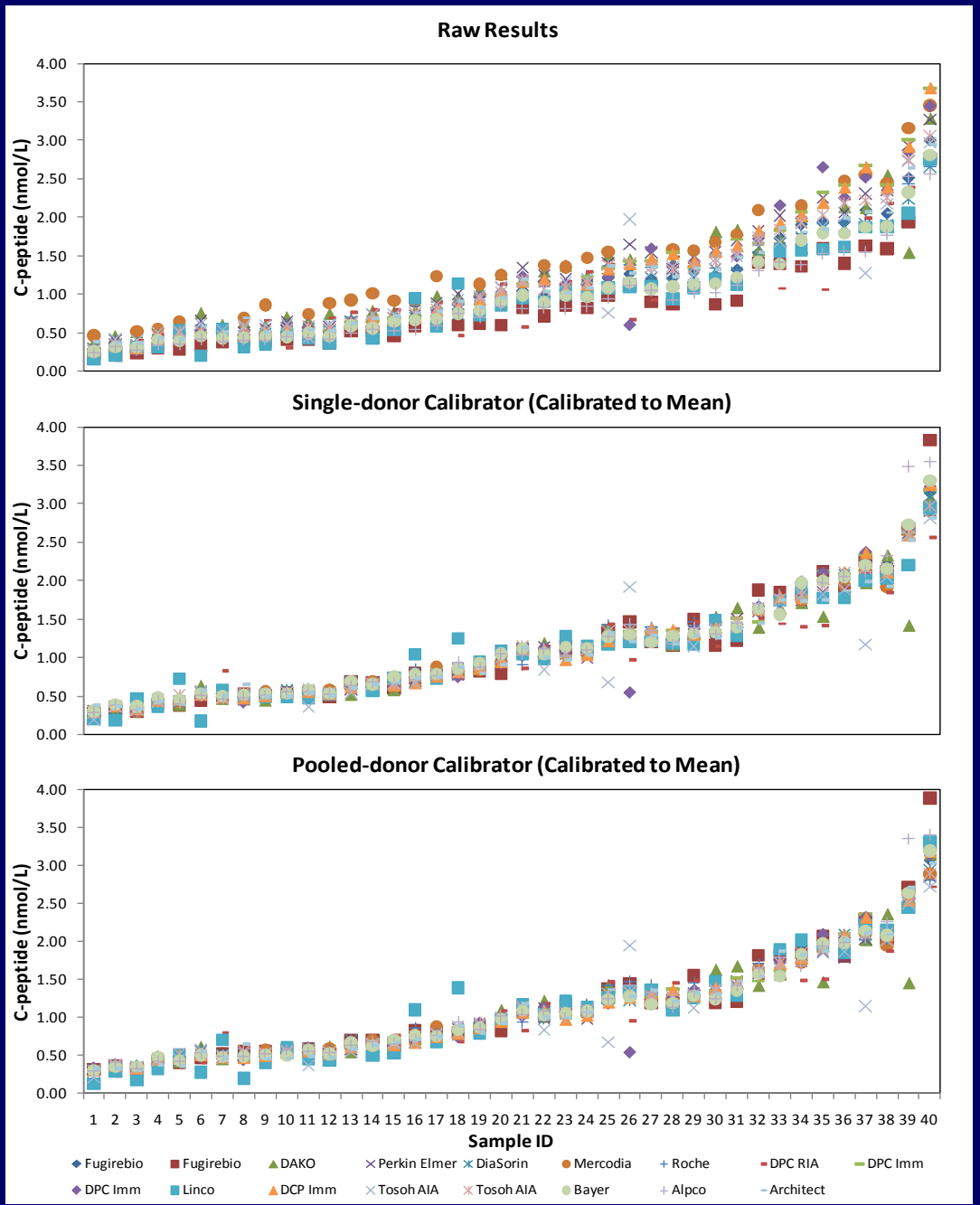
Phase III: Results

- We verified that variability can be reduced by standardization to the Mass Spectrometry Reference Method
- Results show comparable reduction in between-laboratory variability with single and pooled calibrators (there were no significant differences in the mean responses).
- Reference Method results from serum and EDTA+Aprotinin were comparable

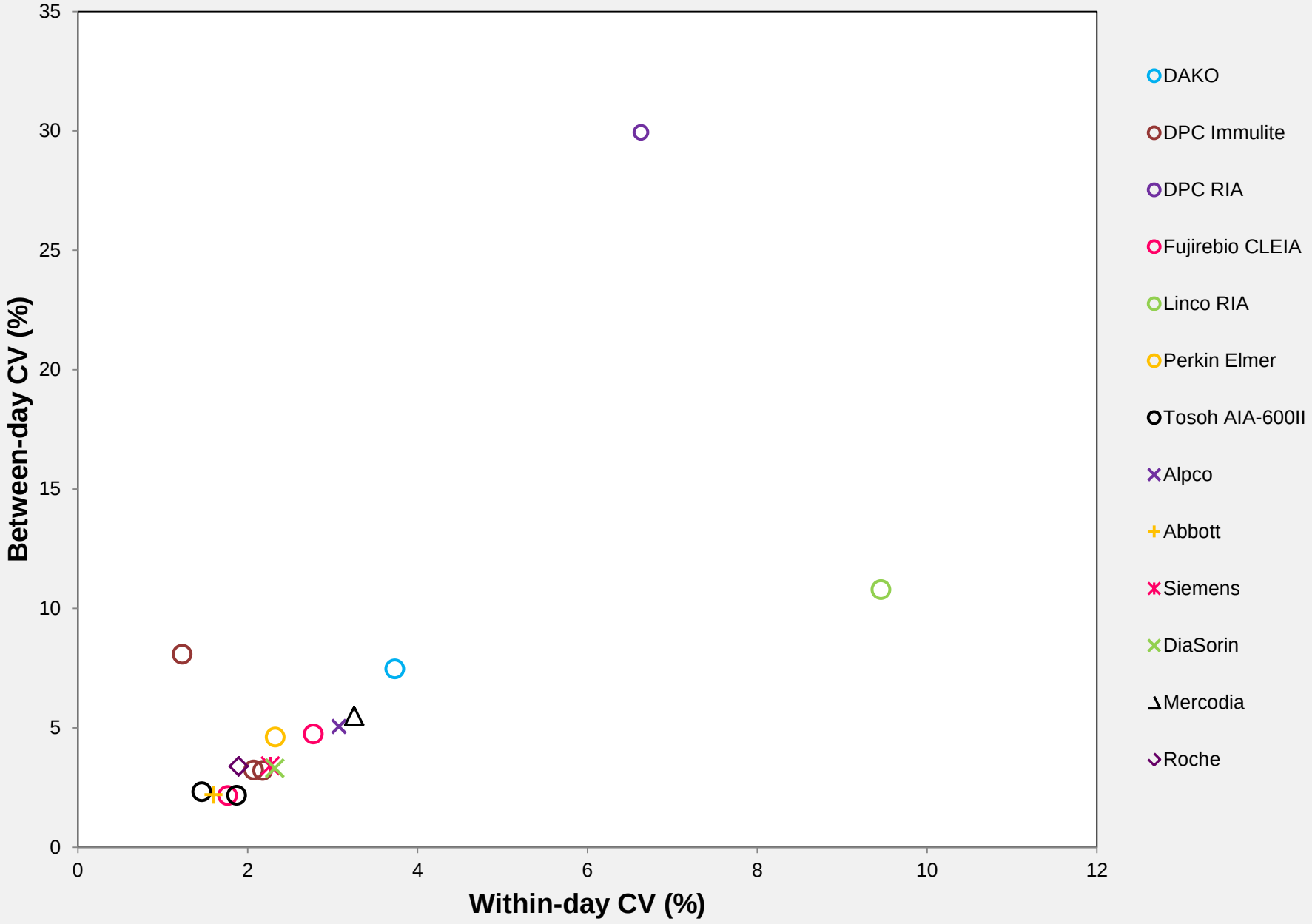
Standardization to a Reference LC-MS Method

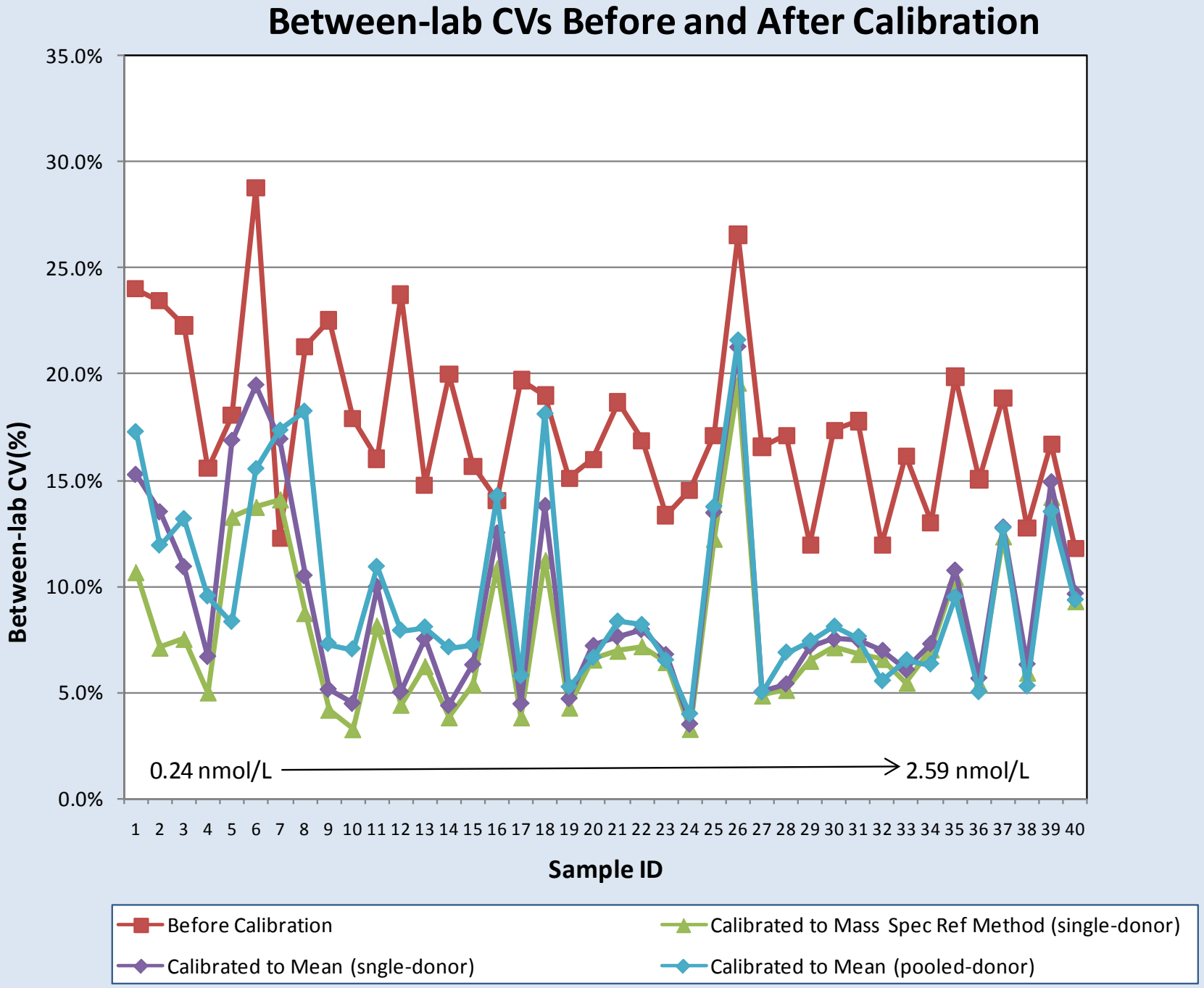


Single vs. Pooled-Donor Calibrator



C-Peptide 2008
Within and Between-day Variability




Between-lab CVs Before and After Calibration
35.0%
30.0%
25.0%
20.0%
15.0%
10.0%
5.0%
0.0%
Between-lab CV(%)
0.24 nmol/L
2.59 nmol/L
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40
Sample ID
Before Calibration
Calibrated to Mass Spec Ref Method (single-donor)
Calibrated to Mean (sngle-donor)
Calibrated to Mean (pooled-donor)

Phase III: Conclusions

- Standardization of C-peptide results to a Mass Spectrometry Reference Method significantly reduces between-lab variability
- Calibrators can be prepared from either single donors or samples pooled from more than one donor
- Serum is acceptable for the Reference Method and can be used for calibrators
- Some methods still have relatively high CVs in some labs

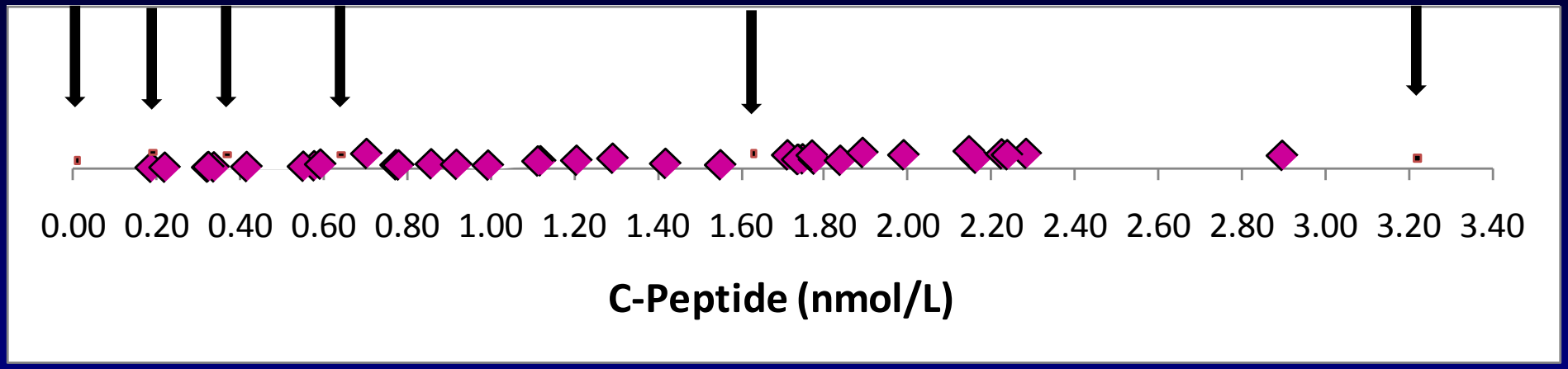
Phase IV

Develop a protocol with manufacturers to evaluate the use of serum calibrators for method re-calibration.

Protocol

- Ship pooled serum calibrators with assigned values and single-donor test samples to all participating manufacturers.
- Manufacturers analyze test samples using their current calibration.
- Manufacturers use the calibrators to “re-calibrate” and then analyze test samples again.
- Compare manufacturer re-calibrated results for samples with Reference Lab results for same.

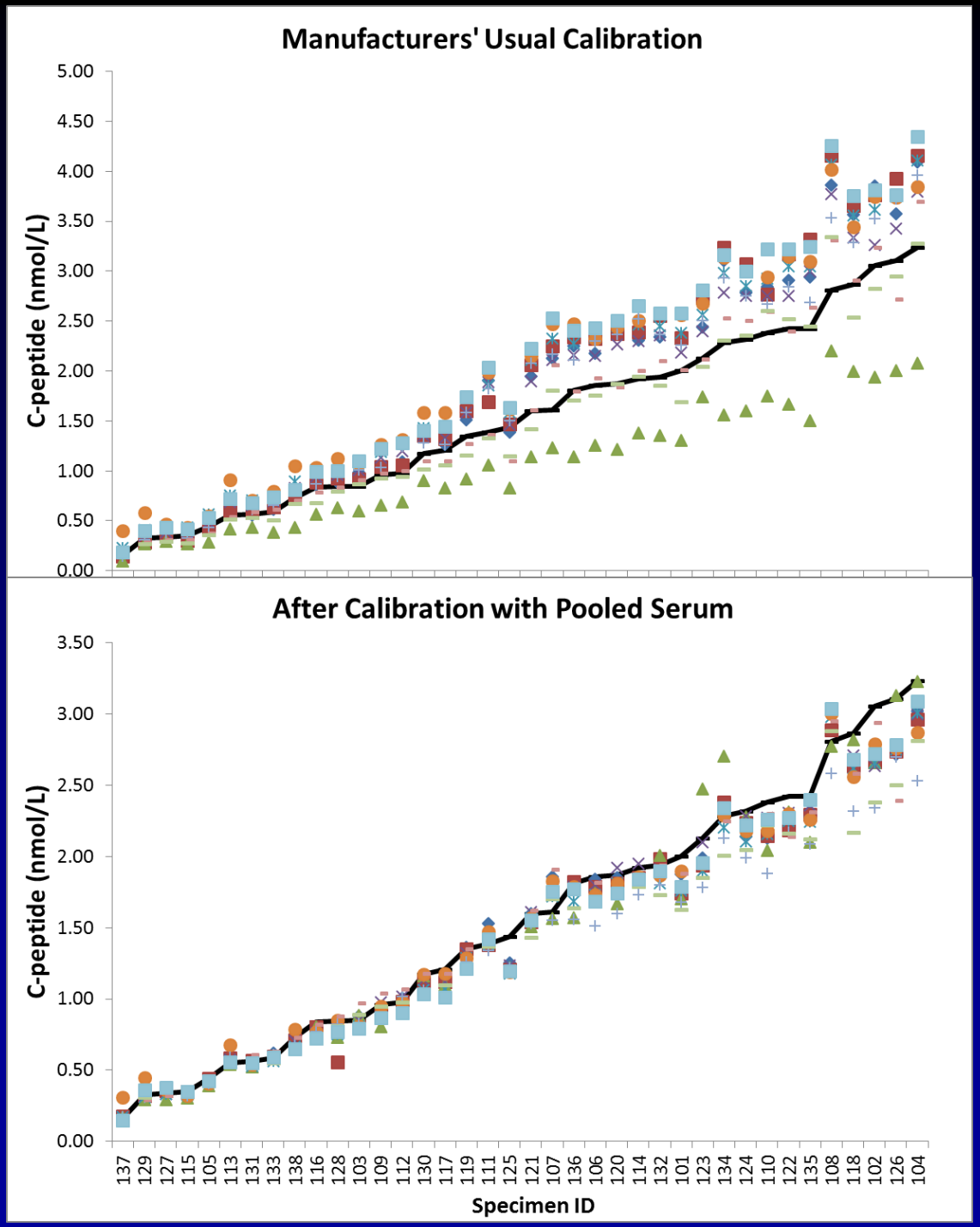
C-Peptide Pooled and Single-donor Sample Range

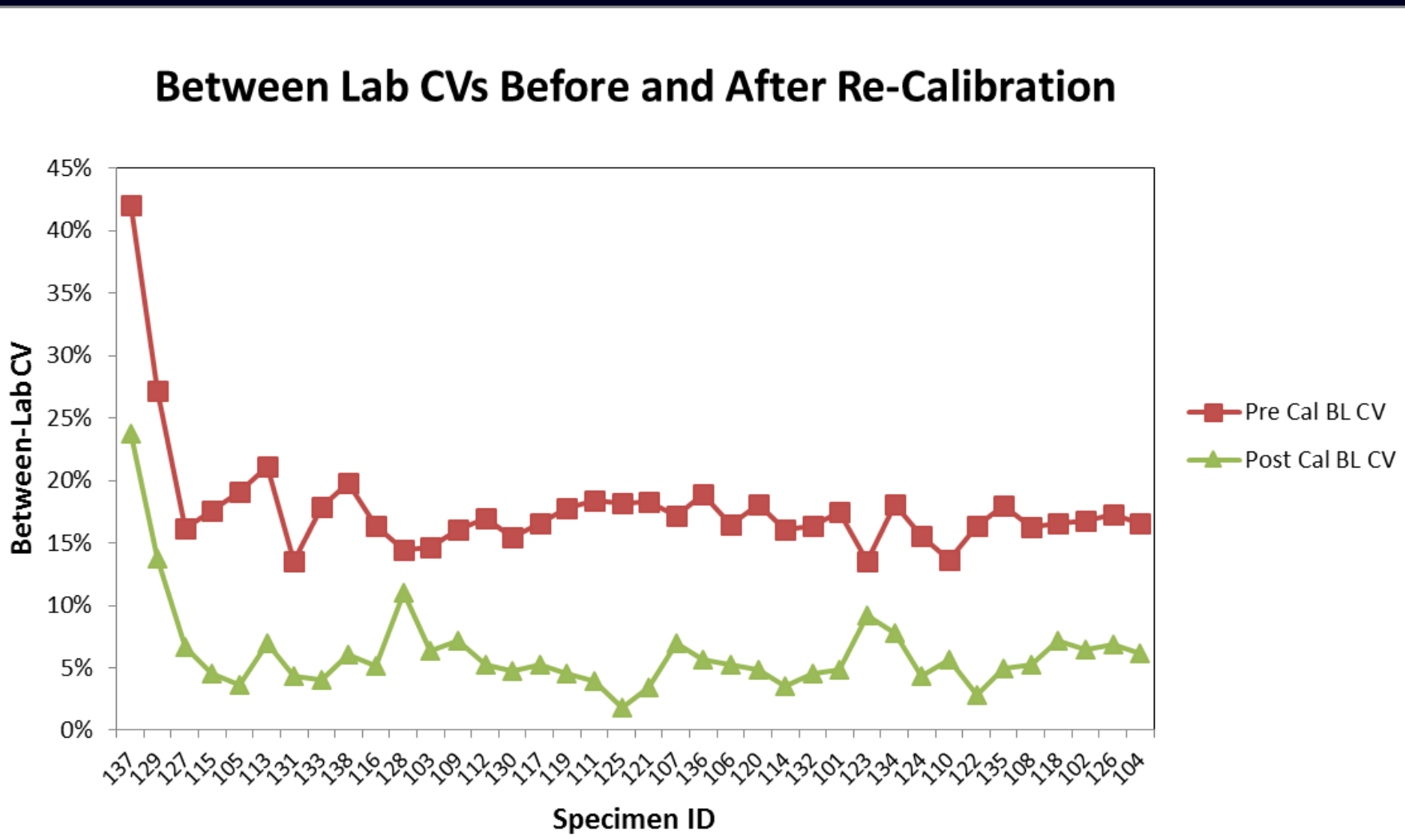


Single-donor samples

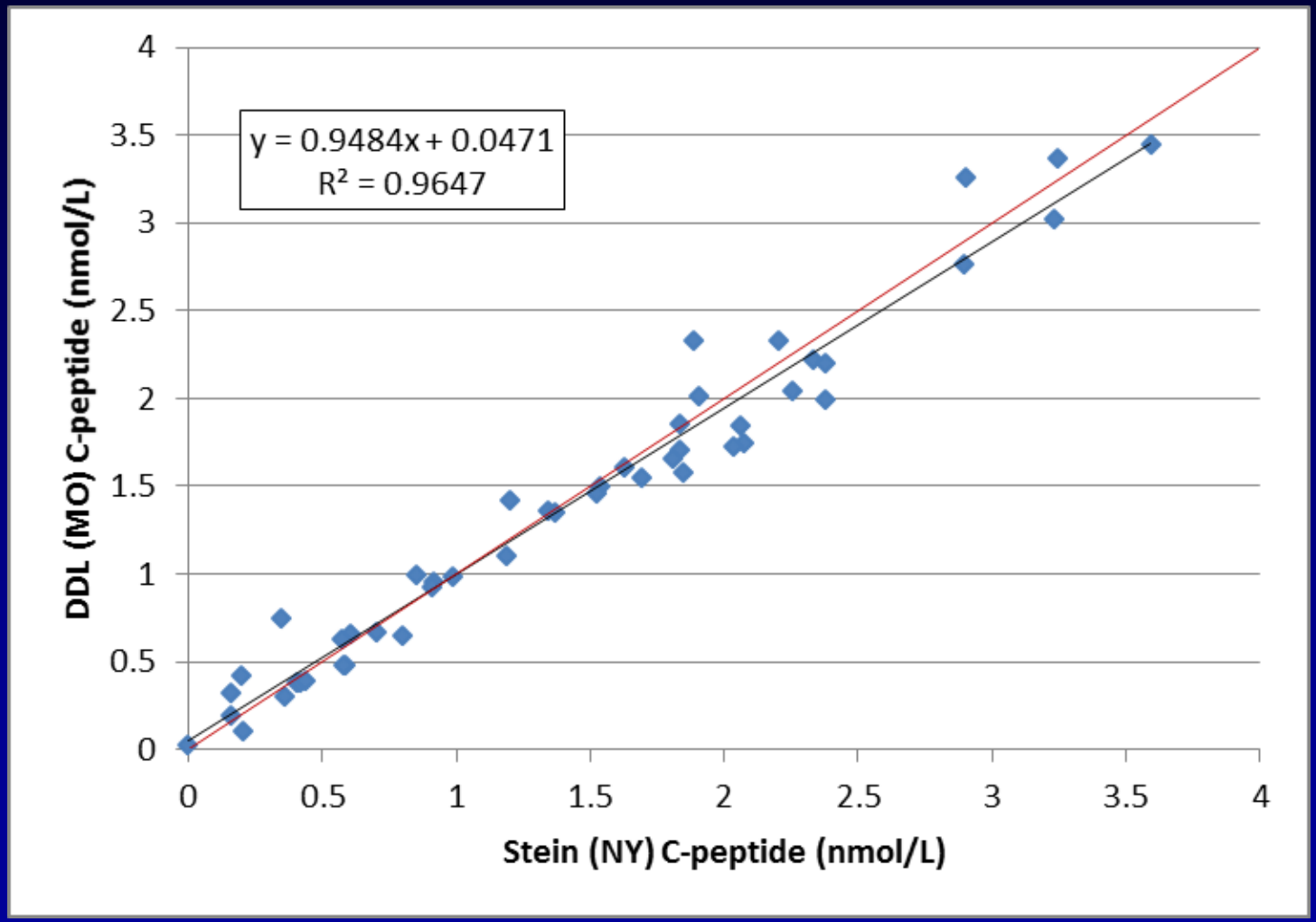
Pooled samples (0.01 to 3.22 nmol/L)

Note: All c-peptide results are based on analysis by LC-MS


Manufacturers' Usual Calibration
C-peptide (nmol/L)
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
After Calibration with Pooled Serum
C-peptide (nmol/L)
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
137 129 127 115 105 113 131 133 138 116 128 103 109 112 130 117 119 111 125 121 107 136 106 120 114 132 101 123 134 124 110 122 135 108 118 102 126 104
Specimen ID


Between Lab CVs Before and After Re-Calibration
Between-Lab CV
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
137 129 127 115 105 113 131 133 138 116 128 103 109 112 130 117 119 111 125 121 107 136 106 120 114 132 101 123 134 124 110 122 135 108 118 102 126 104
Specimen ID
Pre Cal BL CV
Post Cal BL CV

C-Peptide comparison: NY vs MO Reference Labs (2012)



Chromatography
Separation Techniques

Stoyanov et al., J Chromat Separation Techniq 2013, 4:3
http://dx.doi.org/10.4172/2157-7064.1000172

Review Article Open Access

Human C-peptide Quantitation by LC-MS Isotope-Dilution Assay in Serum or Urine Samples

Alexander V Stoyanov[1]*, Shawn Connolly[1], Curt L Rohlfing[1], Eduard Rogatsky[2], Daniel Stein[2] and Randie R Little[1]

[1]*University of Missouri, School of Medicine, Columbia MO, USA*
[2]*Albert Einstein College of Medicine, Yeshiva University, Bronx NY, USA*

Abstract

In this communication we report a simple and efficient approach to C-peptide quantitation using isotope dilution mass-spectrometry analysis. The method facilitates quantitation of C-peptide levels at least one order of magnitude lower compared to concentration levels achieved with an IDA method reported previously. The improvement was due to more intensive sample preparation procedure that, in turn, makes it possible to increase the sample load without a corresponding increase in matrix effects. We also show the results of a comparison study with a second laboratory using a similar previously reported method for C-peptide quantitation.

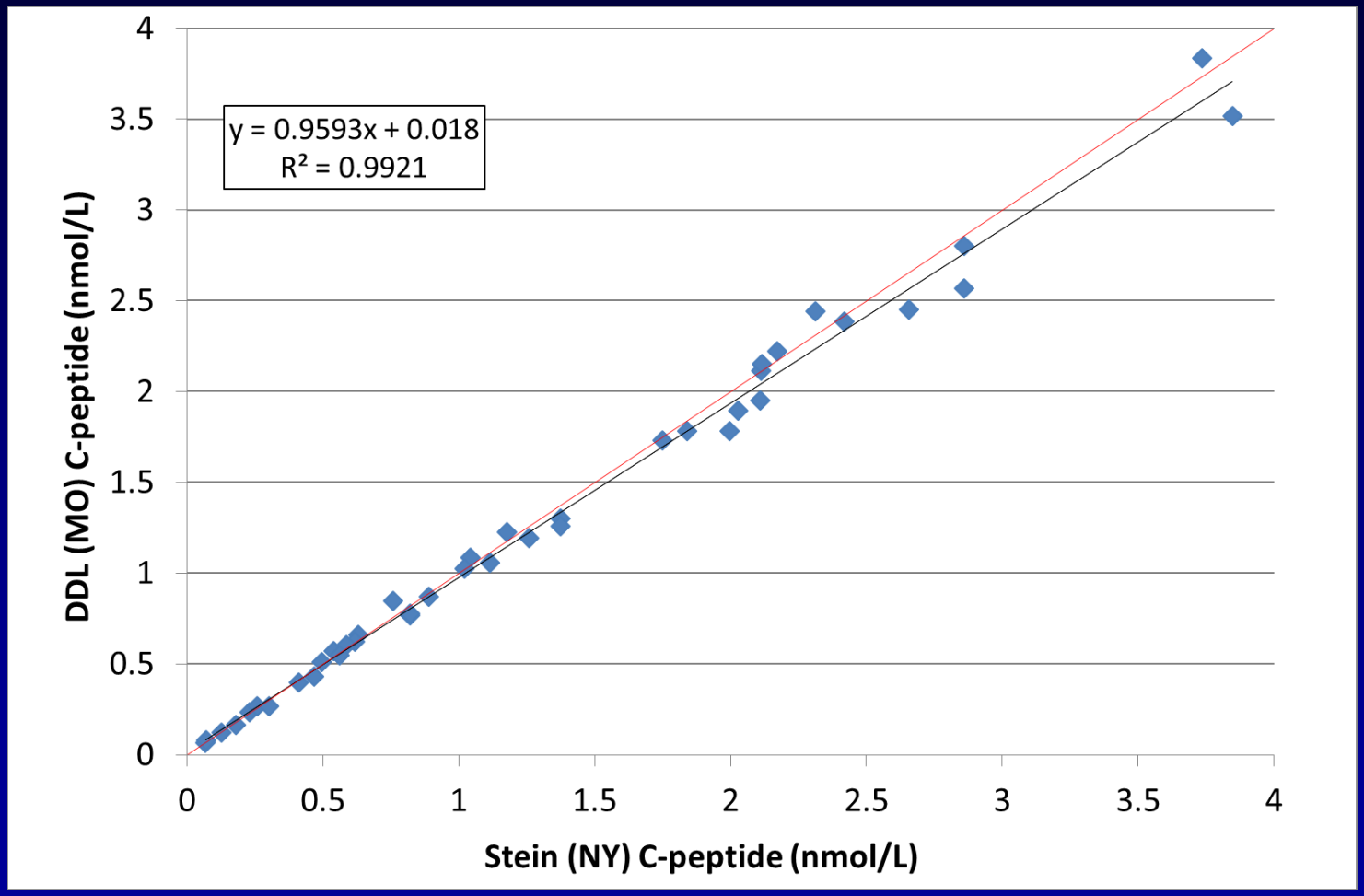
Keywords: C-peptide/Mass spectrometry; Isotope dilution assay; Ion Exchange chromatography; Sample preparation

Introduction

sequentially using different ion-pairing agents [15], which is resulted in different column selectivities. Importantly, the most abundant fragment ion of C-peptide is Y1 [*m/z* 147.1], which represents a yield of only 1.5% using collision induced fragmentation (the standard fragmentation

Analyte	Reference measurement method/procedure	Applicable matrice(s)	Measurement principle/technique	Reference(s)
immunoglobulin G	IFCC Committee on Plasma Proteins (C-PP)	human serum	Optimized immunoturbidimetry/ immunonephelometry	Blirup-Jensen S., Protein standardization III: method optimization. Basic principles for quantitative determination of human serum proteins on automated instruments based on turbidimetry or nephelometry, *Clin. Chem. Lab. Med.*, 2001, 39 (11), 1098-1109
immunoglobulin M	IFCC Committee on Plasma Proteins (C-PP)	human serum	Optimized immunoturbidimetry/ immunonephelometry	Blirup-Jensen S., Protein standardization III: method optimization. Basic principles for quantitative determination of human serum proteins on automated instruments based on turbidimetry or nephelometry, *Clin. Chem. Lab. Med.*, 2001, 39 (11), 1098-1109
a2-macroglobulin	IFCC Committee on Plasma Proteins (C-PP)	human serum	Optimized immunoturbidimetry/ immunonephelometry	Blirup-Jensen S., Protein standardization III: method optimization. Basic principles for quantitative determination of human serum proteins on automated instruments based on turbidimetry or nephelometry, *Clin. Chem. Lab. Med.*, 2001, 39 (11), 1098-1109
C-peptide	UMC DDL reference method for serum C-peptide	lyophilized, fresh, or frozen human serum or urine	Liquid chromatography mass spectrometry (LC/MS)	Use of cation exchange chromatography for human C-peptide isotope dilution - Mass spectrometric assay, Stoyanov AV et al., *J. Chromatogr. A*, 2011, **1218**, 9244-9249; Human C-peptide Quantitation by LC-MS Isotope-Dilution Assay in Serum or Urine Samples, Stoyanov AV et al., *J. Chromat. Separation Techniq.*, 2013, **4**, 172
C-reactive protein	IFCC Committee on Plasma Proteins (C-PP)	human serum	Optimized immunoturbidimetry/ immunonephelometry	Blirup-Jensen S., Protein standardization III: method optimization. Basic principles for quantitative determination of human serum proteins on automated instruments based on turbidimetry or nephelometry, *Clin. Chem. Lab. Med.*, 2001, 39 (11), 1098-1109
transferrin	IFCC Committee on Plasma Proteins (C-PP)	human serum	Optimized immunoturbidimetry/ immunonephelometry	Blirup-Jensen S., Protein standardization III: method optimization. Basic principles for quantitative determination of human serum proteins on automated instruments based on turbidimetry or nephelometry, *Clin. Chem. Lab. Med.*, 2001, 39 (11), 1098-1109

C-Peptide comparison: NY vs. MO Reference Labs (2014)

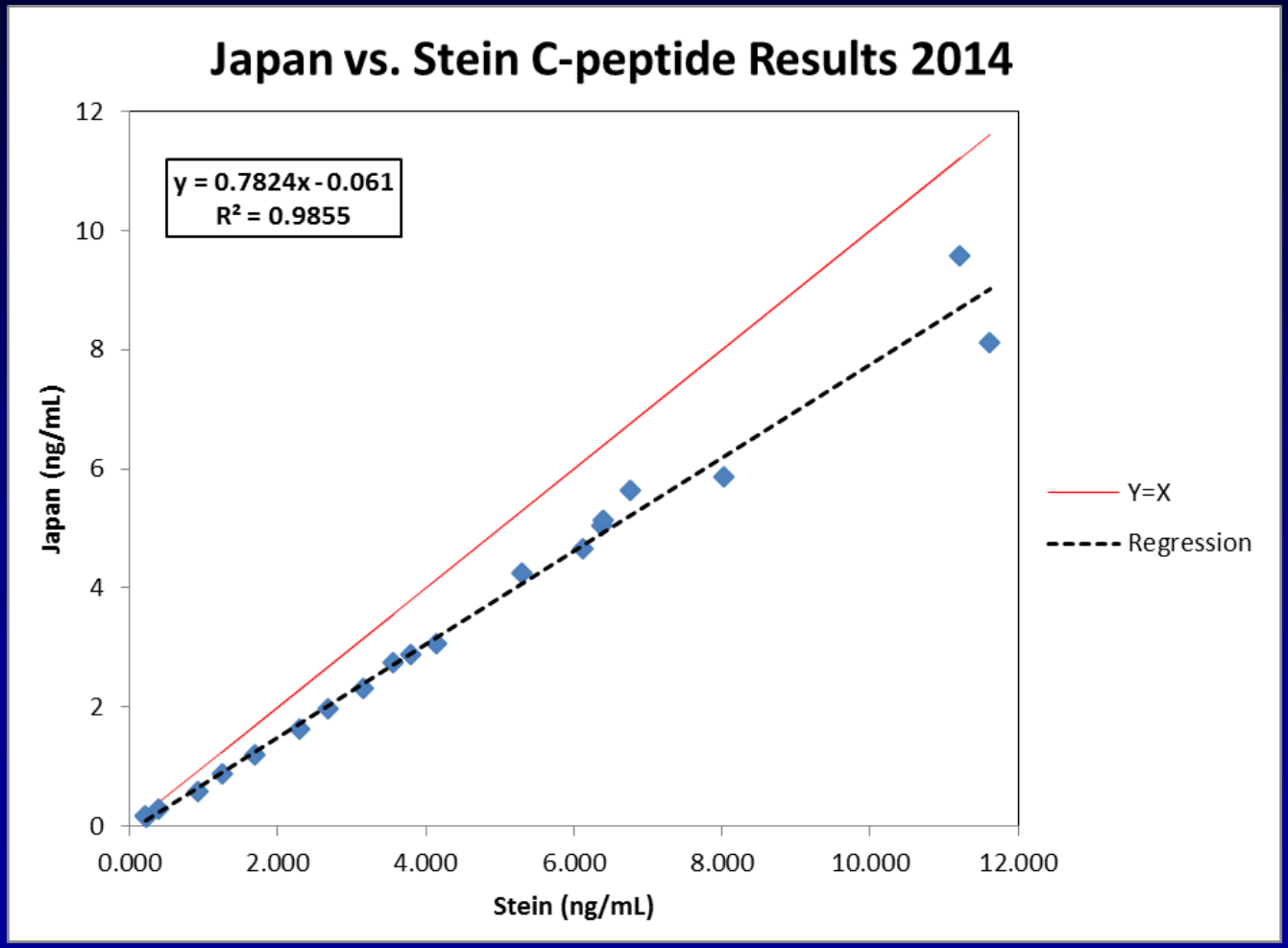


Current Issues

New C-Peptide Reference Materials

- **BIPM - NIM**
  - used for the CCQM-K115 inter-lab comparison
  - not currently available
  - will be JCTLM listed when it becomes an available CRM
  - no comparison with existing (Stein) standard
- **NIBSC**
  - will *not* be listed with JCTLM
  - currently available
  - comparison with existing (Stein) standard possible
- **NMIJ CRM**
  - will be JCTLM listed
  - currently available
  - comparison with NMIJ standard possible; comparison with Japan Reference method/standard completed

Comparison with NMIJ CRM and Japanese Reference Method



There is ~a 25% bias between Japan and US!

Next Steps

- Compare Stein standard with NMIJ Reference Material and NISCB Reference Material at MO lab (DDL)
- Decide which Reference Material to use (JCTLM listed?, Which standards agree?, etc) and adjust calibration accordingly
- Provide revised values to manufacturers for Secondary Reference Materials previously sent and for new materials in preparation
- Recommend re-calibration by manufacturers

Thank you!

Participating Manufacturers:

- Alpco
- Roche
- Siemens
- Tosoh
- Milipore
- DiaSorin
- Mercodia
- Abbott
- Fujirebio

NIDDK C-peptide Standardization Committee:

- Judith Fradkin (NIDDK)
- Randie Little (Univ. of Missouri)
- Greg Miller (Virginia Commonwealth Univ.)
- Gary Myers (AACC)
- Jerry Palmer (Univ. of Washington)
- Kenneth Polonsky (Washington Univ.)
- Lisa Spain (NIDDK)
- Daniel Stein (Albert Einstein College of Med)

Participating Laboratories:

- Paolo Pozzilli, Univ. Campus Bio-Medico (Italy)
- Charlotte Becker, Malmö Univ. Hospital (Sweden)
- Lucilla Monti, San Raffaele Hospital (Italy)
- Merete Frandsen, Thomas Mandrup-Poulsen, Steno Diabetes Center (Denmark)
- Armando Mendez, Linda Jones, Univ. of Miami (FL)
- Jean Bucksa, Vicky Makky, Univ. of Minnesota Medical Center, Fairview (MN)
- Veronica Luzzi, Gene Sherrow, Washington Univ. (MO)
- Liz Rinehart, Linco Diagnostic Services, Inc. (MO)
- Jon Nakamoto, Anne Caston-Balderrama, Quest Diag. - Nichols Institute (CA)
- Vinod Gaur, Northwest Lipid Metab/Diab. Res. Lab., Univ. of Washington (WA)
- Alethea Tennill, University of Missouri (MO)
- Akira Shimada, Taro Maruyama, Keio University (Japan)
- Tetsuro Kobayashi, University of Yamanashi (Japan)
- Kelly Chun, Esoterix Inc. (CA)
- Ralph Jacob, Yale University (CT)
- Dan Stein, Eduard Rogatsky, Albert Einstein College of Med.(NY)
- Anette Ziegler, Kerstin Koczwara, Diabetes Research Institute Munich (Germany)
- Anders Isakson, Mona Landin-Olsson, Lund Univ. (Sweden)
- Spiros Fourlanos, Royal Melbourne Hospital (Australia)
- Bill Roberts, ARUP (UT)
- John DeVore,Abbott (US)
- Patrik Lindstedt, Mercodia (Sweden)
- Thomas Ciesiolka, Roche (Germany)
- Craig LaMarca, ALPCO (US)

Manufacturer	Location	Method
Roche	Germany	Elecsys Modular
Millipore (Linco)	US	ELISA, RIA
DiaSorin	Germany	Liaison
Mercodia	Sweden	ELISA , Ultrasensitive ELISA
Tosoh	Japan	AIA 1800
Alpco	US	ELISA
Siemens	UK	DPC Immulite
Siemens	US	Advia
Abbott	US	Architect
Fugirebio	Japan	Lumipulse