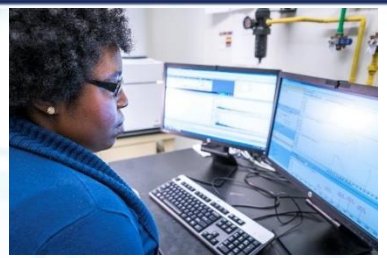


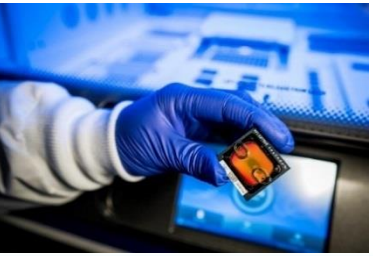


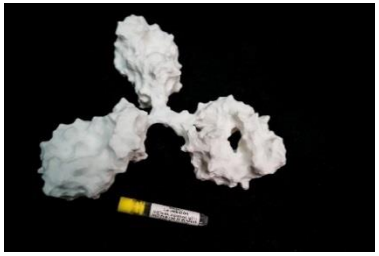
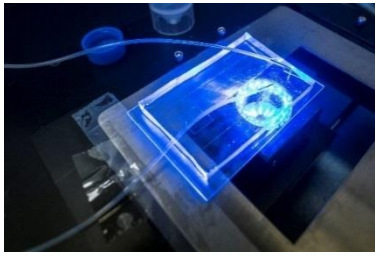
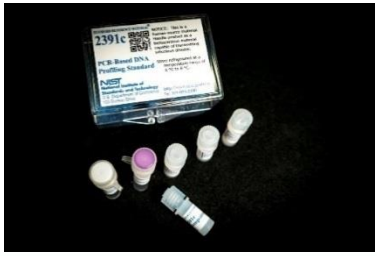
BIOMOLECULAR MEASUREMENT DIVISION

Building and Maintaining Reference Measurement Systems for Kidney Disease Markers

Karen Phinney, Johanna Camara, and Ashley Beasley Green

National Institute of Standards and Technology
Chemical Sciences Division and Biomolecular Measurement Division

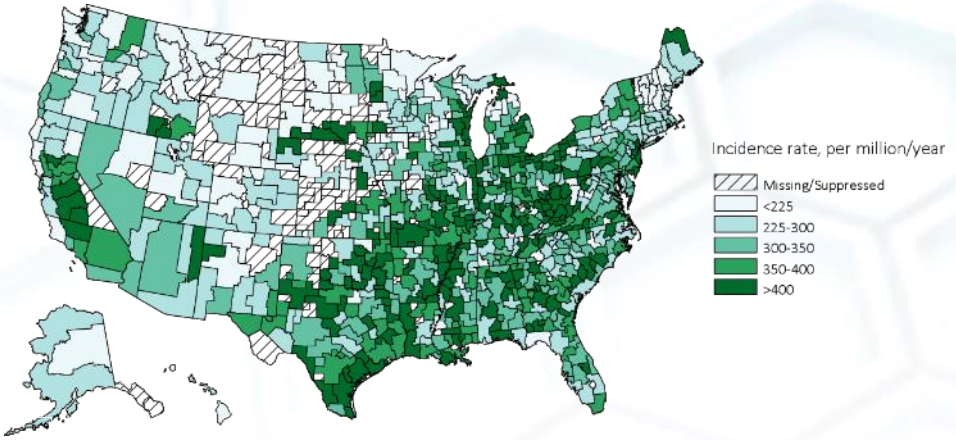


Impact of Renal Disease on U.S. Society

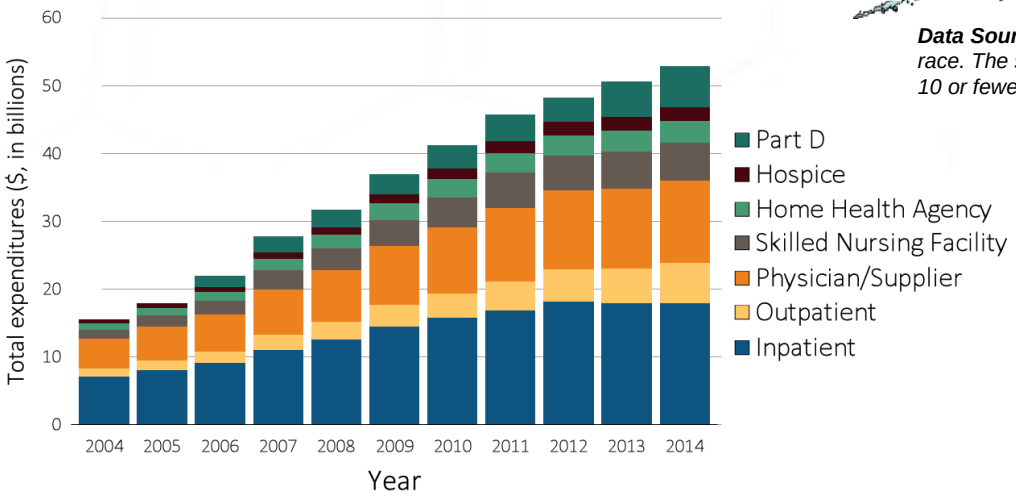
Prevalence of End-Stage Renal Disease (ESRD)

Figure 1.3 Map of the adjusted* incidence rate (per million/year) of ESRD, by Health Service Area, in the U.S. population, 2014



***Data Source**: Special analyses, USRS ESRD Database. *Adjusted for age, sex and race. The standard population was the U.S. population in 2011. Values for cells with 10 or fewer patients are suppressed. Abbreviation: ESRD, end-stage renal disease.*

Figure 6.3 Trends in total Medicare Parts A, B, and D fee-for-service spending for CKD patients aged 65 and older, by claim type, 2004-2014



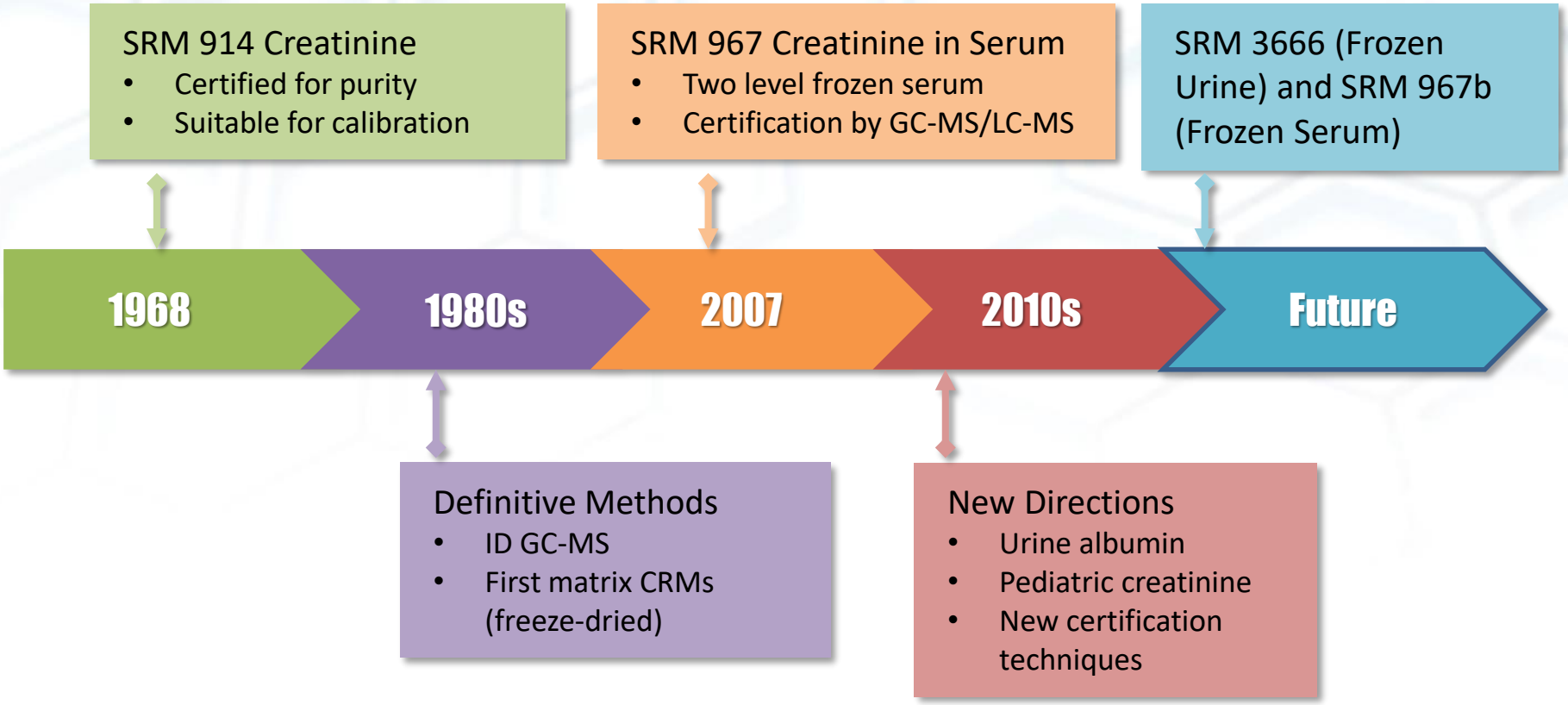
Economic Impact of Kidney Disease

United States Renal Data System. 2019 USRDS annual data report: Epidemiology of kidney disease in the United States. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2019.

Data source: Medicare 5% sample. Part D data was initiated since 2006.

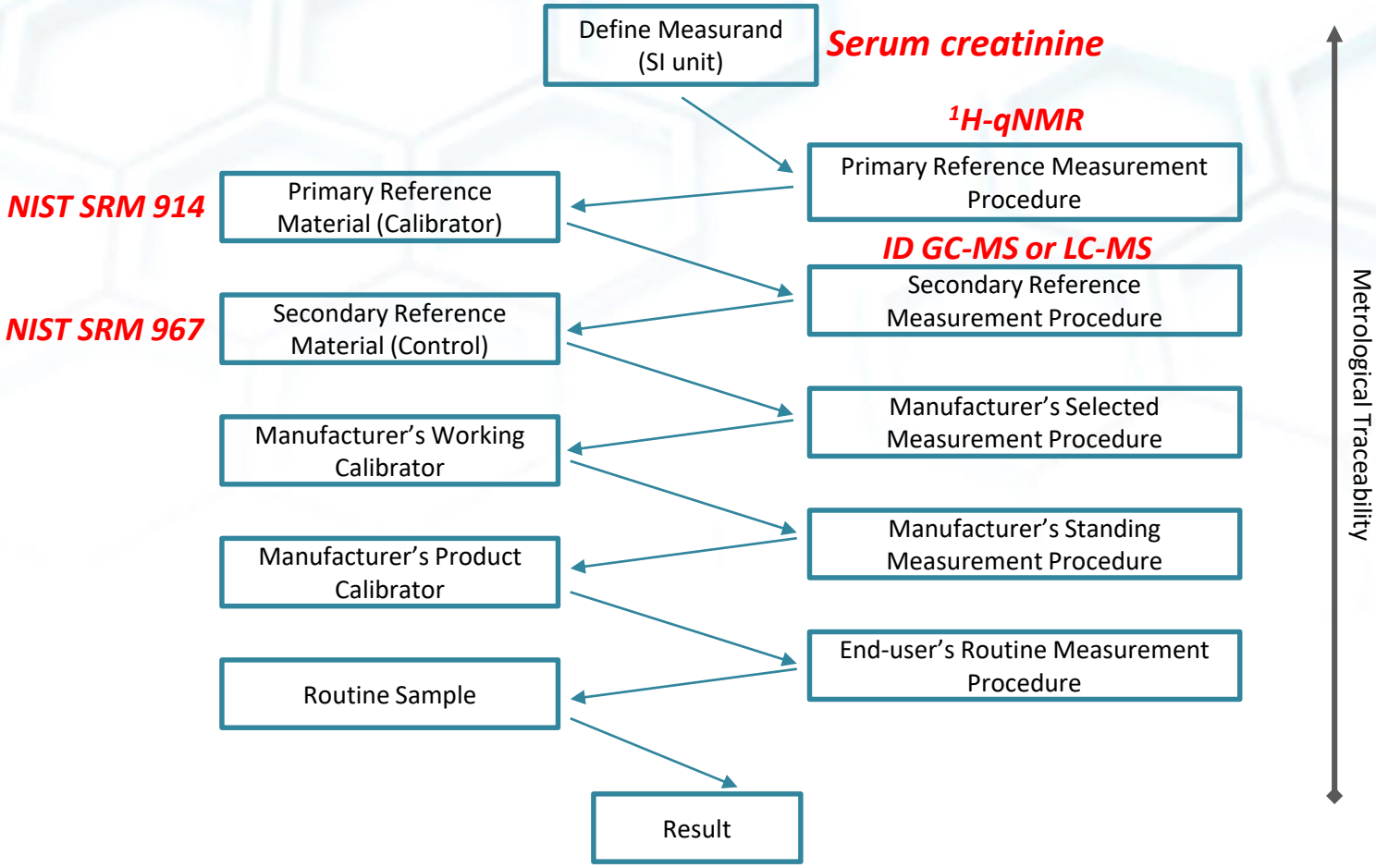
United States Renal Data System. 2016 USRDS annual data report: Epidemiology of kidney disease in the United States. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2016.

History of Kidney Disease Marker Standardization Efforts at NIST



- Development of SRM 967 was in collaboration with the National Kidney Disease Education Program (NKDEP) Laboratory Working Group, later combined with IFCC WG on Standardisation of Albumin Assay in Urine
- With creatinine standardization well underway (in developed countries), LWG began focus on urine albumin measurement and reporting

An Example Reference Measurement System

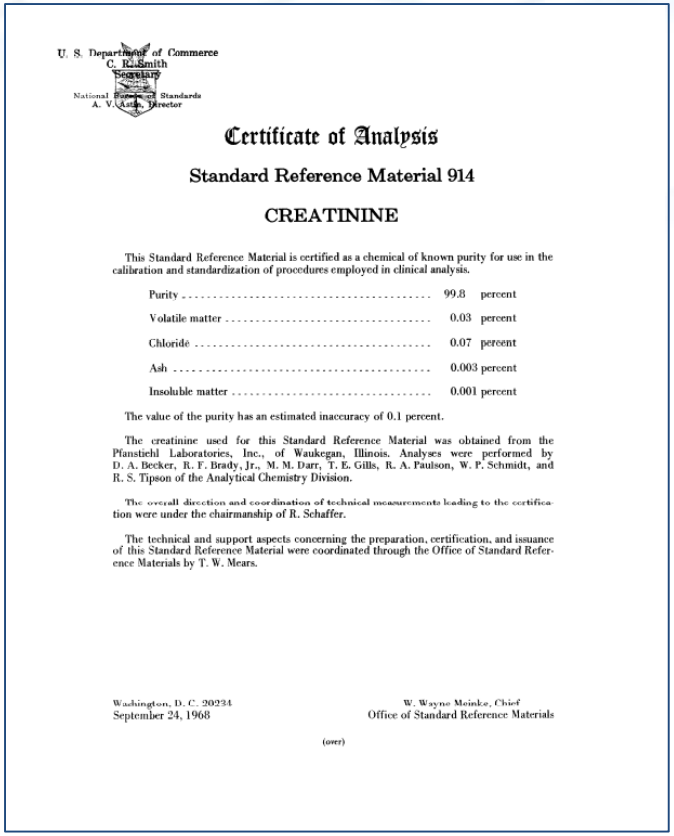


Maintaining the reference measurement system requires maintaining both the reference methods and reference materials (and reference laboratories)

SRM 914 Creatinine

Then: SRM 914

Purity assessment by GC, TLC, ash



U. S. Department of Commerce
C. R. Smith
Secretary
National Bureau of Standards
A. V. Astin, Director

Certificate of Analysis

Standard Reference Material 914

CREATININE

This Standard Reference Material is certified as a chemical of known purity for use in the calibration and standardization of procedures employed in clinical analysis.

Purity	99.8	percent
Volatile matter	0.03	percent
Chloride	0.07	percent
Ash	0.003	percent
Insoluble matter	0.001	percent

The value of the purity has an estimated inaccuracy of 0.1 percent.

The creatinine used for this Standard Reference Material was obtained from the Pfanstiehl Laboratories, Inc., of Waukegan, Illinois. Analyses were performed by D. A. Becker, R. F. Brady, Jr., M. M. Darr, T. E. Gills, R. A. Paulson, W. P. Schmidt, and R. S. Tipson of the Analytical Chemistry Division.

The overall direction and coordination of technical measurements leading to the certification were under the chairmanship of R. Schaffer.

The technical and support aspects concerning the preparation, certification, and issuance of this Standard Reference Material were coordinated through the Office of Standard Reference Materials by T. W. Mears.

Washington, D. C. 20234
September 24, 1968

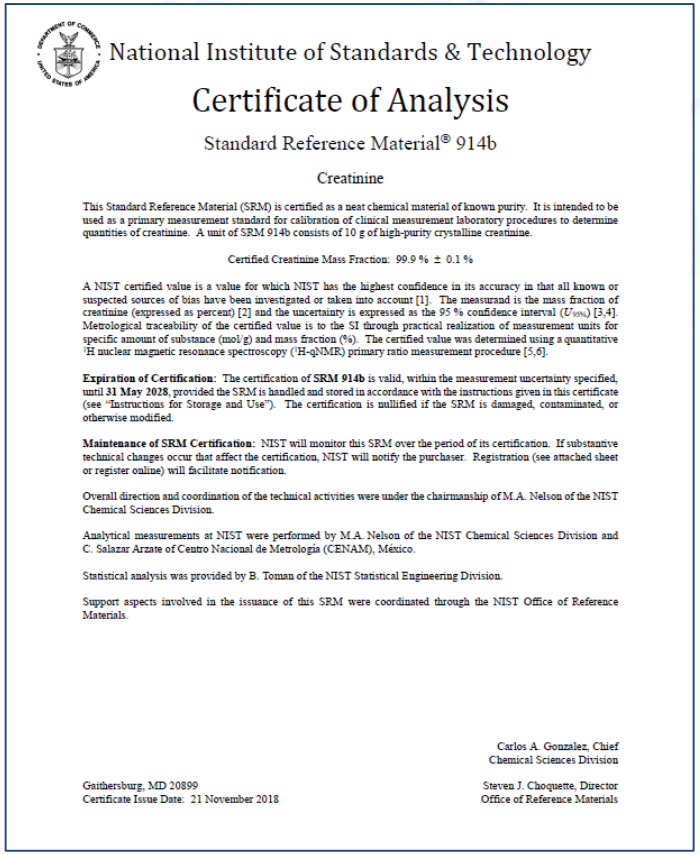
W. Wayne Meinke, Chief
Office of Standard Reference Materials

(over)

"Value of the purity has an estimated inaccuracy of 0.1%"

Now: SRM 914b

Purity assessment by ^{1}H-qNMR



National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 914b

Creatinine

This Standard Reference Material (SRM) is certified as a neat chemical material of known purity. It is intended to be used as a primary measurement standard for calibration of clinical measurement laboratory procedures to determine quantities of creatinine. A unit of SRM 914b consists of 10 g of high-purity crystalline creatinine.

Certified Creatinine Mass Fraction: 99.9 % ± 0.1 %

A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1]. The measurand is the mass fraction of creatinine (expressed as percent) [2] and the uncertainty is expressed as the 95 % confidence interval ($U_{95\%}$) [3,4]. Metrological traceability of the certified value is to the SI through practical realization of measurement units for specific amount of substance (mol/g) and mass fraction (%). The certified value was determined using a quantitative ^{1}H nuclear magnetic resonance spectroscopy (^{1}H-qNMR) primary ratio measurement procedure [5,6].

Expiration of Certification: The certification of **SRM 914b** is valid, within the measurement uncertainty specified, until **31 May 2028**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Instructions for Storage and Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Overall direction and coordination of the technical activities were under the chairmanship of M.A. Nelson of the NIST Chemical Sciences Division.

Analytical measurements at NIST were performed by M.A. Nelson of the NIST Chemical Sciences Division and C. Salazar Arzate of Centro Nacional de Metrología (CENAM), México.

Statistical analysis was provided by B. Toman of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

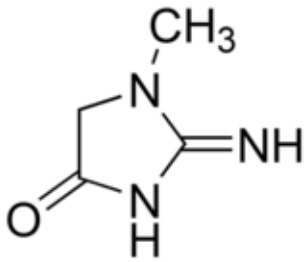
Carlos A. Gonzalez, Chief
Chemical Sciences Division

Gaithersburg, MD 20899
Certificate Issue Date: 21 November 2018

Steven J. Choquette, Director
Office of Reference Materials

"Mass fraction 99.9% ± 0.1%"

Certification of SRM 967 Creatinine in Frozen Human Serum



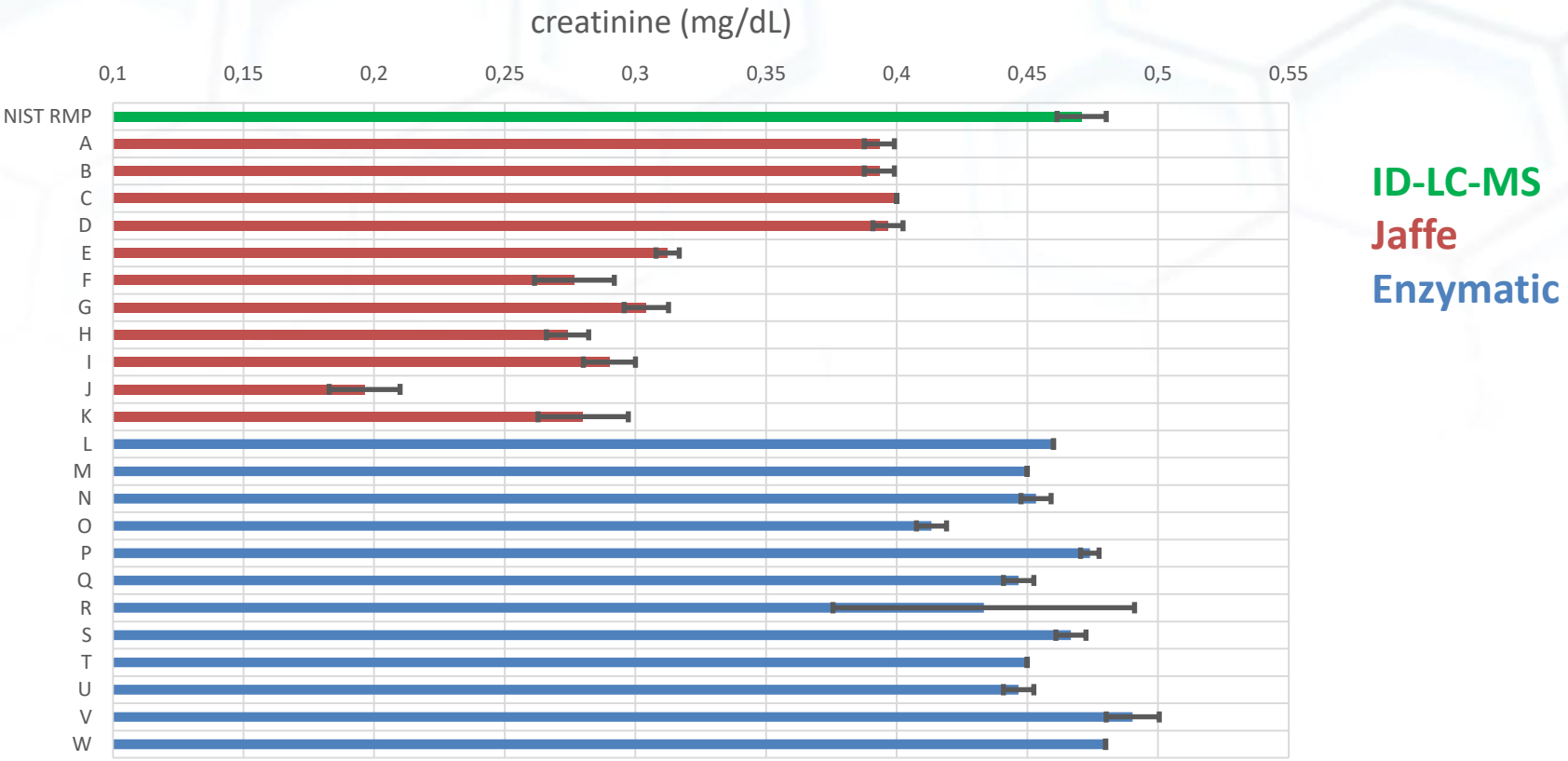
creatinine

	GC-MS Method		LC-MS Method	
	Pool 1 (μmol/L)	Pool 2 (μmol/L)	Pool 1 (μmol/L)	Pool 2 (μmol/L)
Set 1	69.2	345.8	66.2	346.0
	66.6	344.9	66.2	346.9
	67.7	344.9	66.0	345.9
	66.9	343.4	66.1	344.6
			65.9	345.6
Set 2	68.1	346.8	65.9	345.7
	67.2	346.7		
	67.5	348.4	66.1	345.5
	67.1	348.0	66.3	346.4
			66.2	346.1
Set 3	66.4	343.6	65.9	346.3
	66.6	346.5	66.0	346.0
	66.4	346.8	65.9	346.1
	66.4	346.7		
			66.3	347.3
			66.3	347.3
			65.9	347.4
			66.3	347.9
			66.0	346.0
			65.9	347.0

- SRM 967 Issued in 2007 depleted less than 2 years later
- SRM 967a issued in 2009, similar properties
- Transition to LC-MS for RMPs

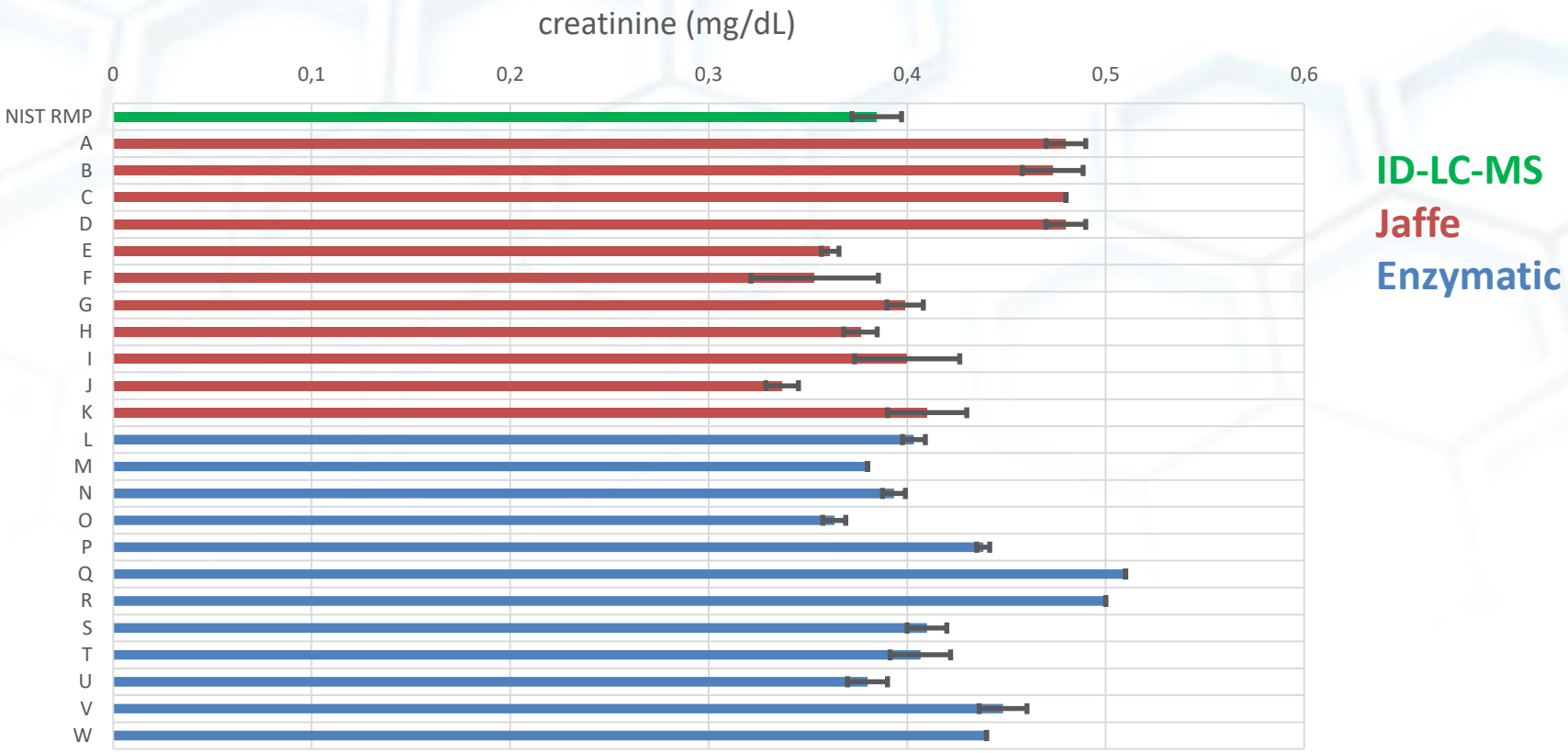
Next Generation SRMs

- Need for kidney disease screening in pediatric population
- Pooling of normal adult sera won't achieve desired concentration (~ 0.4 mg/dL)
- SRM 967a Level 1 is ~ 0.85 mg/dL



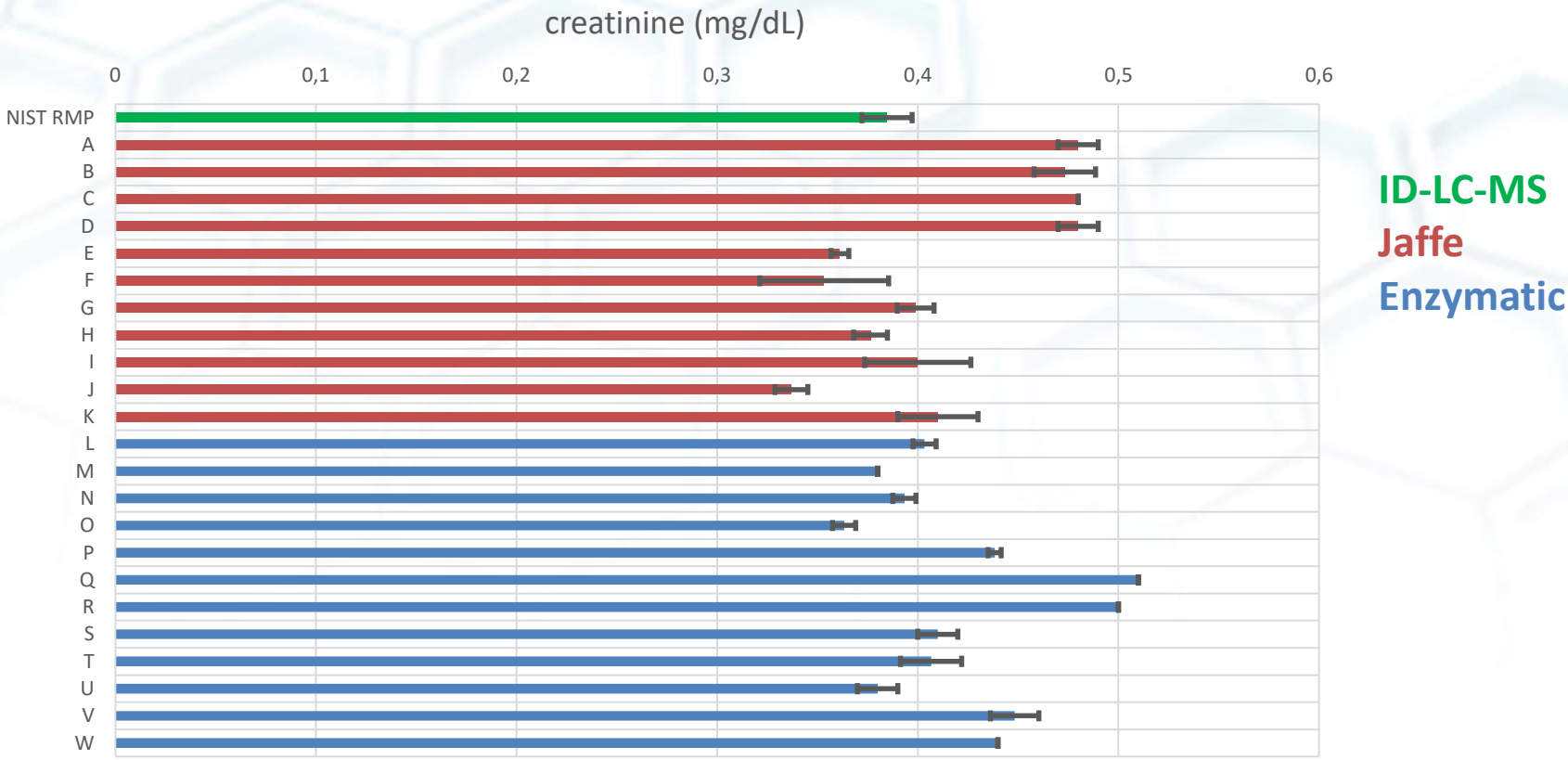
NIST CREATININE 3 (SIGMATRIX ULTRA + SRM 914A CREATININE)

Blended Native + Synthetic Sera



NIST CREATININE 4 (SIGMATRIX ULTRA + SRM 909C FROZEN HUMAN SERUM)

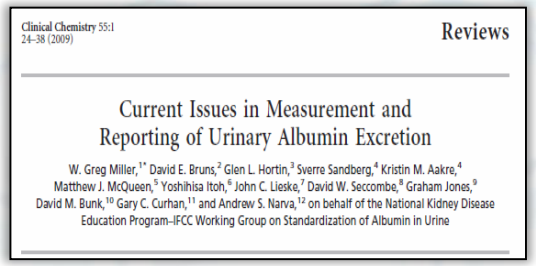
Blended Native + Synthetic Sera



NIST CREATININE 4 (SIGMATRIX ULTRA + SRM 909C FROZEN HUMAN SERUM)

- No ideal synthetic or blended native/synthetic serum could be identified
- Preparation of SRM 967b will be based on native (adult) serum
- Contractors claim they can achieve ~0.4 mg/dL

Standardization of Urine Albumin


Clinical Chemistry 55:1
24–38 (2009)

Reviews

Current Issues in Measurement and Reporting of Urinary Albumin Excretion

W. Greg Miller,[1*] David E. Bruns,[2] Glen L. Hortin,[3] Sverre Sandberg,[4] Kristin M. Aakre,[4] Matthew J. McQueen,[5] Yoshihisa Itoh,[6] John C. Lieske,[7] David W. Seccombe,[8] Graham Jones,[9] David M. Bunk,[10] Gary C. Curhan,[11] and Andrew S. Narva,[12] on behalf of the National Kidney Disease Education Program–IFCC Working Group on Standardization of Albumin in Urine

Metrological Traceability

Define Measurand (SI unit)

Urine Albumin (mg/L)

NIST SRM 2925 (Labeled HSA Material)

Primary Reference Material (Calibrator)

NIST SRM 3666

Secondary Reference Material (Control)

Manufacturer's Working Calibrator

Manufacturer's Product Calibrator

Routine Sample

Result

Primary Reference Measurement Procedure

NIST LC-MS/MS Method

Secondary Reference Measurement Procedure

Manufacturer's Selected Measurement Procedure

Manufacturer's Standing Measurement Procedure

End-user's Routine Measurement Procedure

Beasley-Green A., et al. J Proteome Res. 2014; 13(9): 3930-3939

SRM 2925 Recombinant Human Serum Albumin (Primary Reference Calibrator for Urine Albumin) (Frozen)

Intended Use:

- *Calibration of liquid chromatography-tandem mass spectrometric procedures for the determination of human serum albumin*
- *Value-assignment of NIST SRM 3666, secondary reference material*

Value	Method	Assigned Value
Certified Value (Recombinant HSA Concentration)	**Amino Acid Analysis (ID-MS)**	**0.958 g/L (± 0.0219 g/L)** (NIMJ amino acid CRMs)
Reference Value	Density	1.00016 g/mL (± 0.00001 g/mL)

Protein Qualitative Characterization:

Peptide Profile

DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADESAENCDKSLHTLF

GDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLY

EIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFK

AWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPL

LEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYE

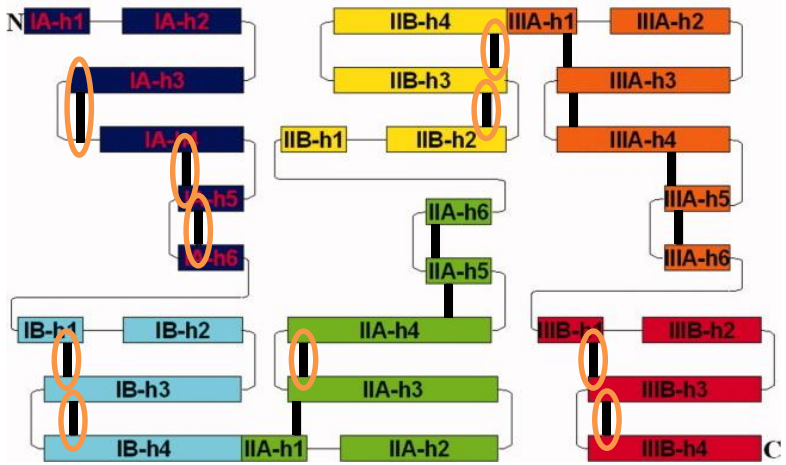
TTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVE

VSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDET

YVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETC

FAEEGKKLVAASQAALGL

Tertiary Structure-Disulfide Profile



Biopolymers, Volume: 97, Issue: 11, Pages: 889-898, First published: 24 May 2012.

NIST Measurement Procedure for Urine Albumin

Journal of proteome research
Article
pubs.acs.org/jpr

Multiplexed LC–MS/MS Assay for Urine Albumin

Ashley Beasley-Green,* Nijah M. Burris, David M. Bunk, and Karen W. Phinney

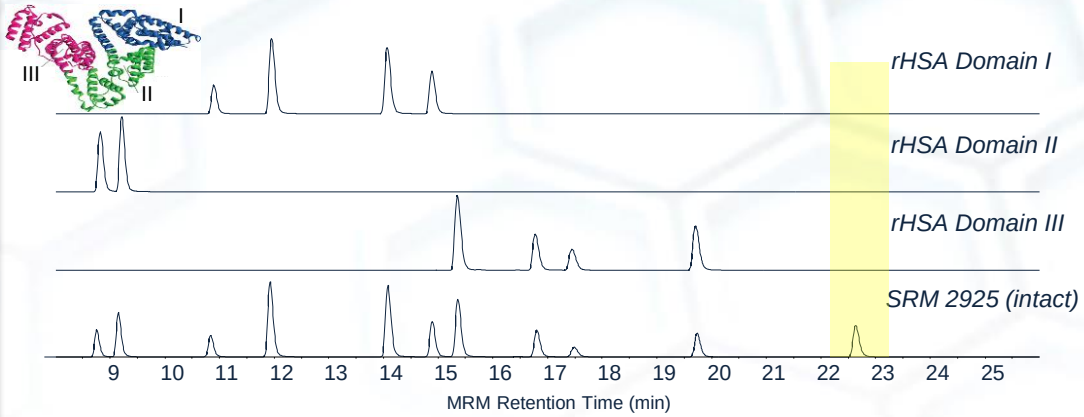
Biomolecular Measurement Division, National Institute of Standards and Technology, Gaithersburg, Maryland 20899-8390, United States

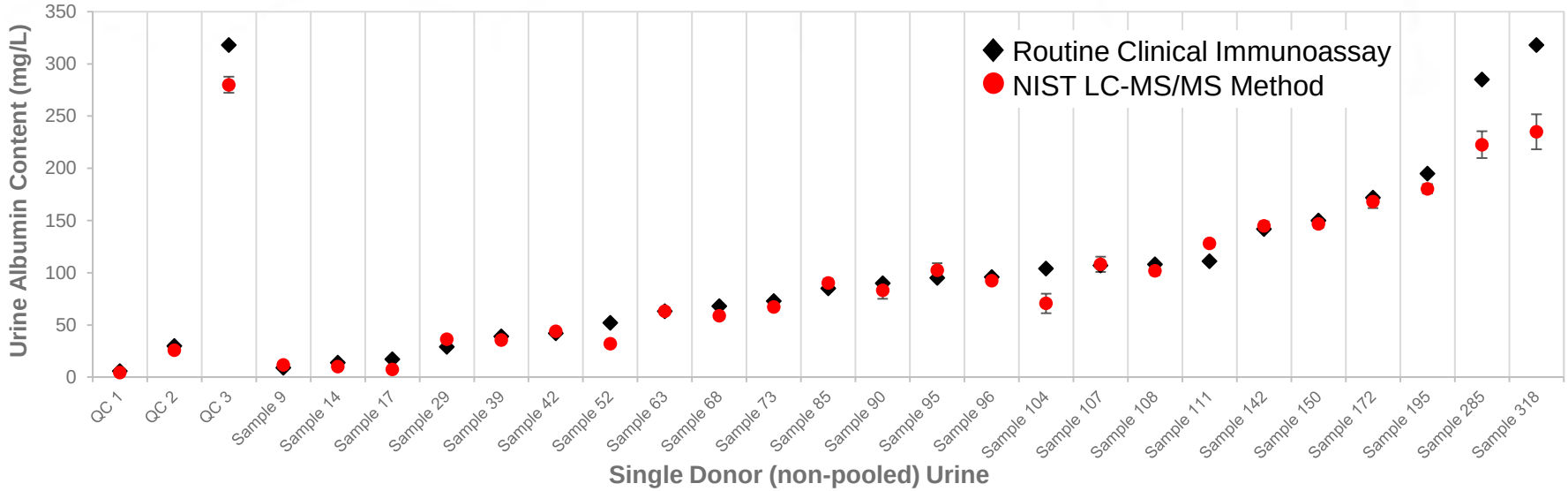
Supporting Information

ABSTRACT: Urinary excretion of albumin is a major diagnostic and prognostic marker of renal dysfunction and cardiovascular disease; therefore, accurate measurement of urine albumin is vital to clinical diagnosis. Although intermethod differences and analyte heterogeneity have been reported for urine albumin measurements, accuracy assessments of the available methods have been hindered by the lack of a reference system, including reference measurement procedures and reference materials, for this clinical analyte. To address the need for a reference measurement system for urine albumin, we have developed a candidate reference measurement procedure that utilizes isotope dilution–mass spectrometry (ID–MS) and multiple reaction monitoring (MRM) to quantify full-length urine albumin in a targeted mass spectrometric-based approach. The reference measurement procedure incorporates an isotopically labeled (^{15}N) full-length recombinant human serum albumin (^{15}N-rHSA) material as the internal standard, which permits the absolute quantitation of albumin in urine. A total of 11 peptides with two transitions per peptide were selected from the tryptic digestion of human serum albumin on the basis of retention time reproducibility, peak intensity, and the degree of HSA sequence coverage. In addition to method validation, the generated calibration curves were used to determine the albumin content in pooled human urine samples to access the accuracy of the MS-based urine albumin quantitation method.

KEYWORDS: *Urine albumin, reference measurement procedure, absolute quantitation, multiple reaction monitoring (MRM), isotope dilution–mass spectrometry (ID–MS)*

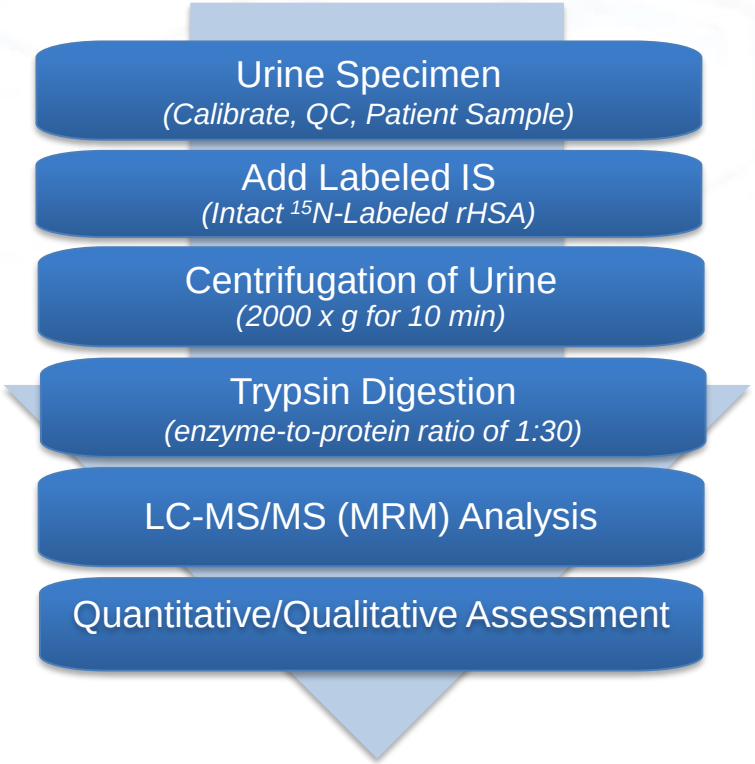
- Multiplexed targeted LC-MS/MS approach
- Purpose: Value-assignment of secondary reference material

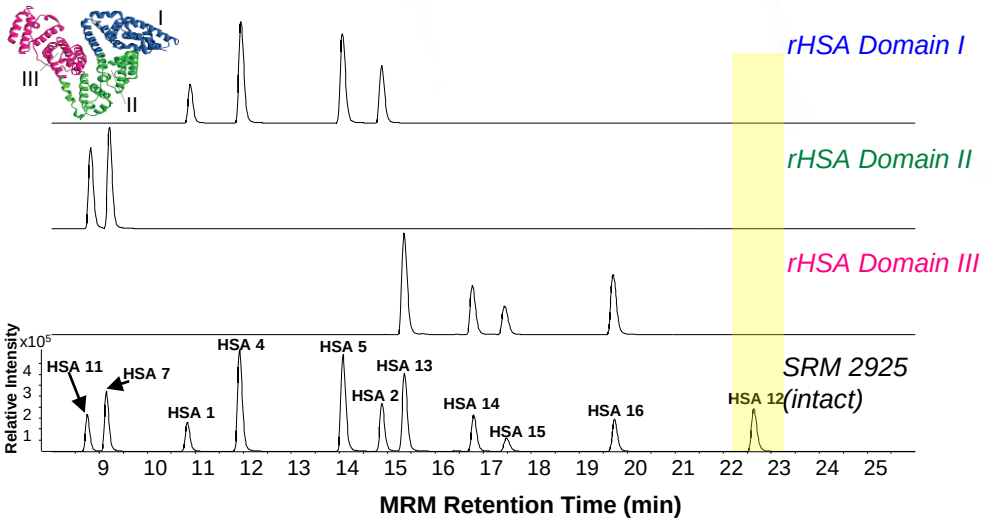




NIST Multiplexed Urine Albumin Method

- Isotope Dilution-Mass spectrometry (ID-MS) targeted approach
- Multiplexed assay that supports quantitative and qualitative assessment of urine albumin
  - *11 peptides that span HSA sequence*
  - *2 transitions per peptide: 23 measurements*





Beasley-Green A., et al. J Proteome Res. 2014; 13(9): 3930-3939

Candidate SRM 3666 Albumin and Creatinine in Frozen Human Urine

Intended Use:

- *Matrix-based quality assessment tool for urine albumin assay manufacturers*

Level	Target Endogenous Urine Albumin Content, mg/L
1	5 mg/L - 10 mg/L
2	20 mg/L – 50 mg/L
3	60 mg/L – 180 mg/L
4	200 mg/L – 600 mg/L

**Preliminary results indicate endogenous urine albumin content of pools are within target ranges.*

Material Specifications:

- Recommendations from Stakeholders
- Single Donor Qualifications
  - *No restrictions on donor age, gender, body mass index, or heath status*
  - *Donor urine screen: Nitrates, Leukocyte esterase, Presence of blood, Urine Albumin*
- Four (4) levels of pooled single donor urine
  - *Pool Criterion: Endogenous Urine Albumin*

Material Certified Values:

- Urine Albumin
  - *NIST-developed Multiplexed Urine Albumin LC-MS/MS Measurement Procedure*
- Urine Creatinine
  - *NIST-developed ID-MS LC-MS/MS Method for Creatinine in Urine*

Conclusions

- ✓ Seek industry and clinician input early
- ✓ Know the potential impact of standardization efforts on medical practice
- ✓ Recognize that field is evolving – biomarkers and clinical decision points can change
- ✓ Standardization doesn't end with development of reference methods or materials

Acknowledgments

- National Kidney Disease Education Program (NKDEP) Laboratory Working Group (Greg Miller, Chair)
- IFCC WG-SAU (Lorin Bachmann, Chair)
- Virginia Commonwealth University (Greg Miller and Lorin Bachmann)
- Mayo Clinic and University of Minnesota
- Michael Nelson, NIST Chemical Sciences Division

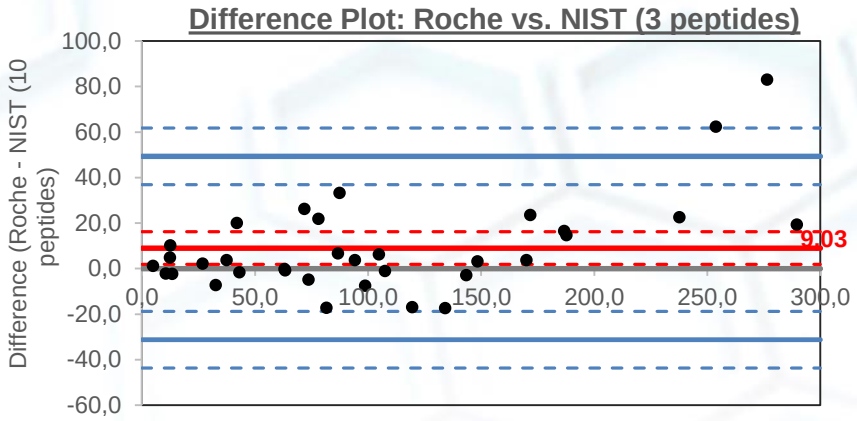
For more information:

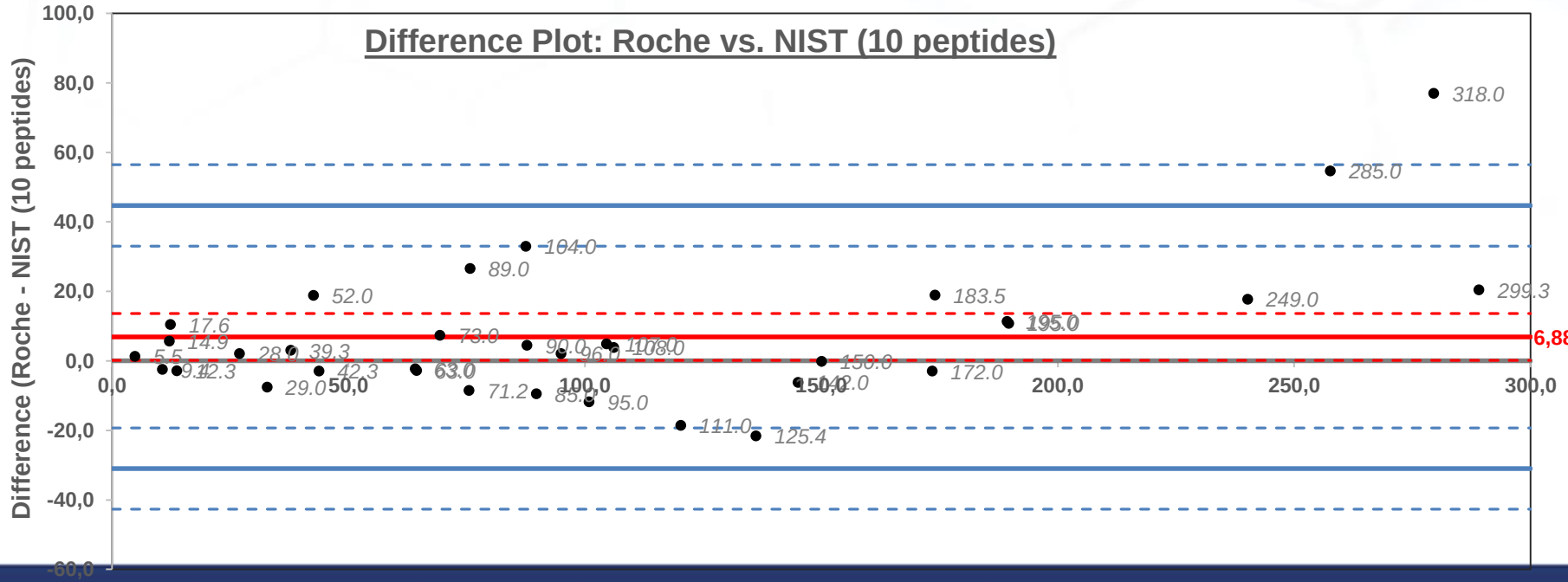
www.nist.gov/srm
karen.phinney@nist.gov

COMPARISON OF URINE ALBUMIN METHODS

Collaboration with Mayo Clinic

- Slight statistical difference between two methods
- Slight decrease in measurements via NIST method compared to Roche (on average)
  - *10-peptide system: decrease of 6.88*
  - *3-peptide system: decrease of 9.03*



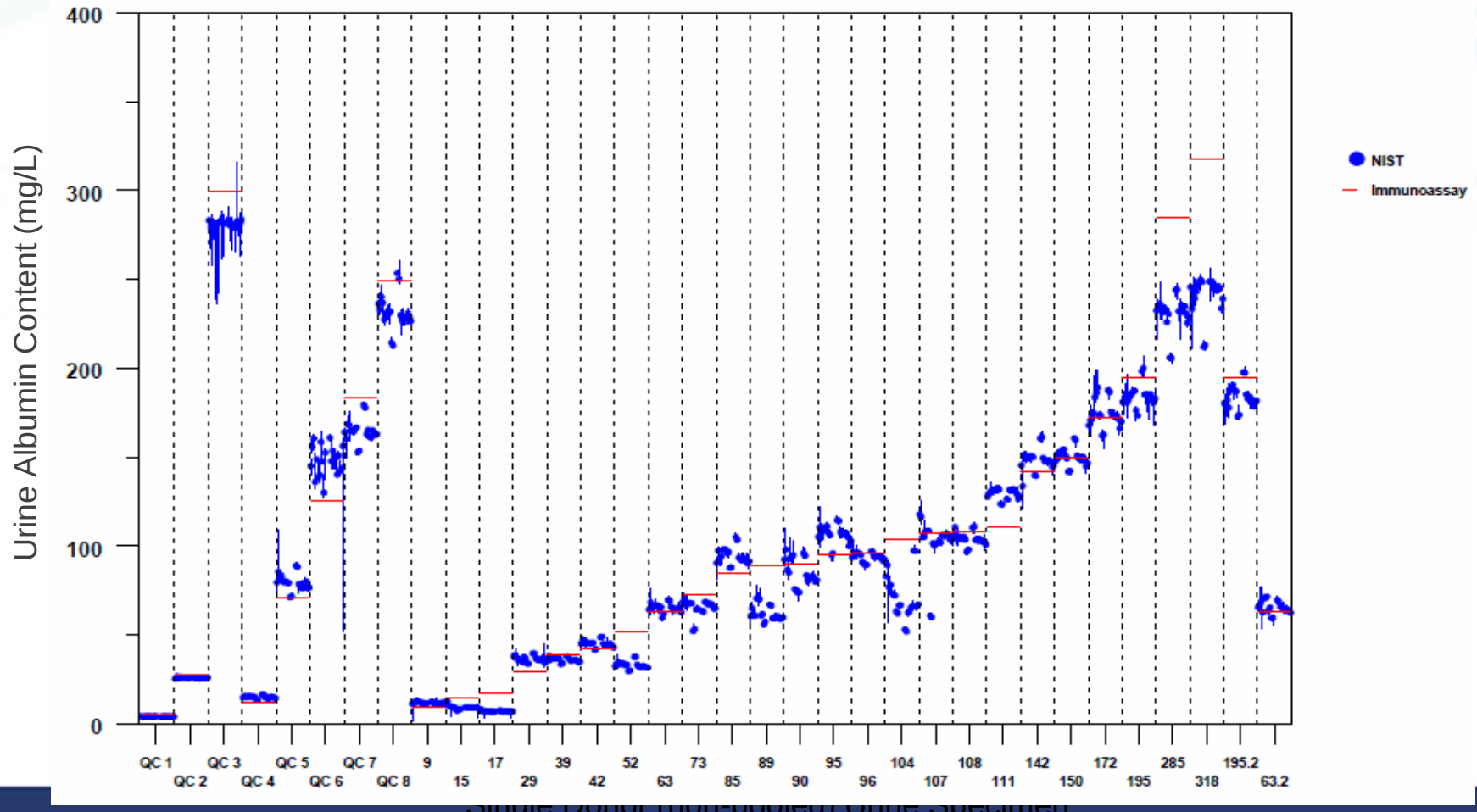


COMPARISON OF URINE ALBUMIN METHODS

Collaboration with Mayo Clinic

NIST Multiplexed LC-MS/MS Assay *(Quantitative and Qualitative Information)*

n=4



Single Donor (non-pooled) Urine Specimen