Building and Maintaining Reference Measurement Systems for Kidney Disease Markers

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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


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

Economic Impact of Kidney Disease

SRM 914 Creatinine
- Certified for purity
- Suitable for calibration

SRM 967 Creatinine in Serum
- Two level frozen serum
- Certification by GC-MS/LC-MS

SRM 3666 (Frozen Urine) and SRM 967b (Frozen Serum)

1968 - 2007
- Definitive Methods
  - ID GC-MS
  - First matrix CRMs (freeze-dried)

2010s
- New Directions
  - Urine albumin
  - Pediatric creatinine
  - New certification techniques

Future

- 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

Define Measurand (SI unit)

Primary Reference Material (Calibrator)

Secondary Reference Material (Control)

Manufacturer’s Working Calibrator

Manufacturer’s Product Calibrator

Routine Sample

Result

Serum creatinine

$^{1}H$-qNMR

Primary Reference Measurement Procedure

Secondary Reference Measurement Procedure

Manufacturer’s Selected Measurement Procedure

Manufacturer’s Standing Measurement Procedure

End-user’s Routine Measurement Procedure

NIST SRM 914

NIST SRM 967

Metrological Traceability

Maintaining the reference measurement system requires maintaining both the reference methods and reference materials (and reference laboratories)
Then: SRM 914

Purity assessment by GC, TLC, ash

“Value of the purity has an estimated inaccuracy of 0.1%”

Now: SRM 914b

Purity assessment by $^1$H-qNMR

“Mass fraction 99.9% ± 0.1%”
## Certification of SRM 967 Creatinine in Frozen Human Serum

### GC-MS Method

<table>
<thead>
<tr>
<th>Pool 1 (µmol/L)</th>
<th>Pool 2 (µmol/L)</th>
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</thead>
<tbody>
<tr>
<td>Set 1</td>
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</tr>
<tr>
<td>69.2</td>
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<td>67.5</td>
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### LC-MS Method

<table>
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<tbody>
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</table>

- 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)
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

**Define Measurand (SI unit)**

**Primary Reference Material (Calibrator)**

**Secondary Reference Material (Control)**

**Manufacturer’s Working Calibrator**

**Manufacturer’s Product Calibrator**

**Routine Sample**

**Primary Reference Measurement Procedure**

**Secondary Reference Measurement Procedure**

**Manufacturer’s Selected Measurement Procedure**

**Manufacturer’s Standing Measurement Procedure**

**End-user’s Routine Measurement Procedure**

**Result**

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**Urine Albumin (mg/L)**

**NIST SRM 2925** (Labeled HSA Material)

**NIST SRM 3666**

Metrological Traceability

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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

<table>
<thead>
<tr>
<th>Value</th>
<th>Method</th>
<th>Assigned Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Certified Value (Recombinant HSA Concentration)</td>
<td>Amino Acid Analysis (ID-MS)</td>
<td>0.958 g/L (± 0.0219 g/L) (NIMJ amino acid CRMs)</td>
</tr>
<tr>
<td>Reference Value</td>
<td>Density</td>
<td>1.00016 g/mL (± 0.00001 g/mL)</td>
</tr>
</tbody>
</table>

Protein Qualitative Characterization:
Peptide Profile

DAHKSEVAHRFKDLGEENFKALVLTAFQYQLQQCPFEDHVKLVNEYTFAKTCVADESAENCDKSLHTLF
GDKLCTVATLREYSEMADCCAKQPERNECFLQHK0DNPPLPRLPFEVDVMCTAFHDEETFLKYLY
ETARRHPYFYAPELLFFAKYKAAFFTECCQAADKAKCLLVPDRLIDRDEF65SKASSAKQRKASLQKFGERAFFK
AWAYASLSQRPKAFAEVSKVLTDILTVEHTECHSDLLLECGADDRADLAncENQDSS5SKLECEKHL
LEKSHCTADEVNEMPADLPSLAADFVEKVDVCKNYAEKDYLGMFLYAYARHPDYSVYLVLLRATYVE
TTTEKCCAAAMHCAYAKVFDFKRLVEEPQLNQKLQNCLEFQFL7EKYKFNQALNLLVYTVKFPQVSTTPLVE
VSRNL6KV56KCKKHPEAKRMPCAEDYLSVVLNQLCIVHEKTPVDVYTKCOTESLVNRRPFCFSALVEDET
YVPEEFNAETFTHADICLSTSEKRIKQQTALVLELKHPKATKEQLKAVMDDDFAAFVEKCALLKETC
FAE65KLVAA5QALGL

Tertiary Structure-Disulfide Profile

NIST Measurement Procedure for Urine Albumin

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

Multiplexed LC–MS/MS Assay for Urine Albumin
Ashley Beasley-Green, Nijah M. Burris, David M. Bunk, and Karen W. Phinney

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Supporting Information

Abstract: Urinary excretion of albumin is a major diagnostic and prognostic marker of renal dysfunction and cardiovascular disease. Therefore, accurate measurement of urinary albumin is vital to clinical diagnosis. Although intermethod differences and analyte heterogeneity have been reported for urine albumin measurements, accurate assessments of the available methods have been hindered by the lack of a reference system, including reference measurement procedures and reference materials for the clinical analysis. To address this need for a reference measurement system for urinary 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 urinary albumin via targeted mass spectrometric-based approach. The reference measurement procedure incorporates an isotopically labeled (15N) full-length recombinant human serum albumin (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 assess the accuracy of the MS–based urinary albumin quantitation method.

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

Routine Clinical Immunoassay
NIST LC-MS/MS Method
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

**Urine Specimen**
(Calibrate, QC, Patient Sample)

**Add Labeled IS**
(Intact $^{15}$N-Labeled rHSA)

**Centrifugation of Urine**
(2000 x g for 10 min)

**Trypsin Digestion**
(enzyme-to-protein ratio of 1:30)

**LC-MS/MS (MRM) Analysis**

**Quantitative/Qualitative Assessment**

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Candidate SRM 3666 Albumin and Creatinine in Frozen Human Urine

Intended Use:
• Matrix-based quality assessment tool for urine albumin assay manufacturers

Material Specifications:
• Recommendations from Stakeholders
• Single Donor Qualifications
  • No restrictions on donor age, gender, body mass index, or health 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

<table>
<thead>
<tr>
<th>Level</th>
<th>Target Endogenous Urine Albumin Content, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5 mg/L – 10 mg/L</td>
</tr>
<tr>
<td>2</td>
<td>20 mg/L – 50 mg/L</td>
</tr>
<tr>
<td>3</td>
<td>60 mg/L – 180 mg/L</td>
</tr>
<tr>
<td>4</td>
<td>200 mg/L – 600 mg/L</td>
</tr>
</tbody>
</table>

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

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

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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)  

Study #3

Single Donor (non-pooled urine specimen)

n=4