Laboratory and Measurement Issues

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Outline

• Serum/plasma creatinine
• Serum/plasma cystatin C
• Urine albumin
• Urine protein
By mid 2010, all creatinine methods will have calibration traceable to isotope dilution mass spectrometry (IDMS) reference measurement procedures

- Some exceptions with minor influence

From a survey of global IVD manufacturers (June 2009)
eGFR reporting: CAP Survey of approximately 4000 participants
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Numeric values reported for eGFR (mL/min/1.73m²)

- <60
- <90
- <120
- All

Percent of laboratories

- 2007
- 2008
- 2009
Specificity of creatinine methods

Calibration traceability to IDMS does not change the influence of interfering substances

- Drugs
- Endogenous substances, e.g.
  - Ketoacidosis
  - Bilirubin
  - Hemoglobin
  - Protein
No consensus recommendations for method specificity requirements

• Both enzymatic and Jaffé (alkaline picrate) methods are influenced by interfering substances

• Enzymatic methods have fewer interfering substance influences than Jaffé

• IFCC and NKDEP are collaborating to compare results for a panel of 389 patient sera and 40 spiked sera containing a wide range of potentially interfering substances
Specificity of creatinine methods

Preliminary data from IFCC/NKDEP evaluation of sera from subjects with interfering substances

• Three Jaffe and four enzymatic methods vs. IDMS reference method

• Both Jaffe and enzymatic methods have influence from interfering substances

• The magnitude of influence for a given substance is different among Jaffe vs. enzymatic methods

• The same substance interfered with some methods (Jaffe or enzymatic) but not others
Outline

• Serum/plasma creatinine

• **Serum/plasma cystatin C**

• Urine albumin

• Urine protein
Current limitation in using cystatin C

- Results do not agree among methods
  - eGFR equations have been proposed but:
    - Limited to the method used to develop the equation
    - Not validated in large populations
Standardization of cystatin C

IFCC work group (chair: A. Grubb)

- Primary reference preparation (PRP)
  - Pure recombinant human Cystatin C

- Secondary reference preparation (SRP)
  - PRP added to delipidated, stabilized human serum pool
  - Characterization and value assignment complete
  - Commutability validation underway
  - To be available in 2010 from Institute for Reference Methods and Materials (IRMM - EU) as ERM-DA 471/IFCC
• Commutable means a standard reference material has a numeric relationship between two, or more, methods equivalent to that observed for clinical samples.

• Tracing calibration to a non-commutable RM will cause mis-calibration for patient samples.
Numeric relationship for patients

Method A (RMP if available) vs. Method B

95% prediction interval

Clinical Specimen
Commutable if same as patients

Method A (RMP if available)

Method B

Clinical Specimen

RM Commutable

95% prediction interval
Not-commutable if different than patients

95% prediction interval
Cystatin C eGFR equation

IFCC work group

• Plans to perform a multi-site evaluation of a new equation for eGFR using standardized methods
Outline

- Serum/plasma creatinine
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- Urine albumin
- Urine protein
Standardization of urine albumin and creatinine measurement and reporting

NKDEP/IFCC conference held in March 2007

Albumin in urine is heterogeneous

- Large and small fragments exist in plasma and urine
- C- and N-terminal truncation occurs
- Tubular uptake is receptor mediated – influences enrichment of modified plasma forms in urine (e.g. glycated)
- Many ligands are concentrated in urine and bind to albumin
- Proteolytic degradation and chemical modifications may occur in tubules, bladder and urine after collection
Albumin measurement procedures

- Immunoassays
  - Primarily nephelometric and turbidimetric procedures
  - Influenced by:
    - Epitope(s) recognized by the antibodies
    - Ab reactivity with modified forms of albumin
  - Polyclonal assays are reactive with some modified albumin forms
Immunoassay precision

CAP Survey, pooled human urine supplemented with albumin, within method comparison

Among laboratory CV, %

Peer group mean, mg/L
Immunoassay vs LC-MS

Average difference = 24%
(N = 92 patient urines)

Albumin measurement procedures

• HPLC assays (size exclusion)
  – Does not resolve albumin from other co-eluting urine proteins causing overestimation
  – Hypothesis of “non-immunoreactive albumin” likely related to non-specificity of HPLC
Immunoassay and HPLC vs LC-MS

Immunoassay

\[ y = 1.03701x - 0.07521 \]
\[ R^2 = 0.9767 \]

HPLC

\[ y = 0.8976x + 0.39026 \]
\[ R^2 = 0.9593 \]

### Immunoassay and HPLC for predicting cardiovascular events

<table>
<thead>
<tr>
<th></th>
<th>Areas under ROC Curves</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Immunoassay</td>
<td>HPLC</td>
</tr>
<tr>
<td>All Participants (N = 5,358)</td>
<td>0.612 (0.586 - 0.638)</td>
<td>0.581 (0.535 - 0.609)</td>
</tr>
<tr>
<td>With Diabetes (N = 1,992)</td>
<td>0.593</td>
<td>0.564</td>
</tr>
<tr>
<td>Without Diabetes (N = 3366)</td>
<td>0.612</td>
<td>0.574</td>
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</table>

McQueen et al. Am J Kidney Dis 2006 Dec;48:889-96
State of the art: results reporting

- A variety of reporting systems:
  - Albumin concentration (e.g. mg/L)
  - Albumin excretion rate (AER, mg/24 h)
  - Albumin/creatinine ratio (ACR)
    - SI (molar) and non-SI units
      - mg/mmol
      - mg/g

- A variety of decision points with different numbers
Recommendations: implement now

- Albumin concentration (mg/L) is difficult to interpret and should not be reported alone
  - Problem for dipsticks
- Albumin/Creatinine ratio should always be reported
  - “mg/mmol” or “mg/g” should be used uniformly in a country or region
Recommendations: urine albumin under development

- Develop a reference method (LC-MS)
- Develop reference standard materials
- Clarify adsorption to containers
- Clarify biological variability
- Clarify molecular forms to measure
- Clarify current immunoassay performance
Outline

- Serum/plasma creatinine
- Serum/plasma cystatin C
- Urine albumin
- Urine protein
Proteins in Urine

- Albumin
- Others
  - Immunoglobulins
  - Bence-Jones
  - Tamm-Horsfall
  - Lysozyme
  - Myoglobin
  - Hemoglobin
  - Bacterial origin
  - Peptides
Quantitative urine protein methods

In order of clinical lab market share in USA:

- Pyrogallol red (dye binding)
- Pyrocatechol violet (dye binding)
- Benzethonium chloride (denaturation/turbidimetry)
- Biuret with precipitation (peptide bonds)
- Coomassie blue (dye binding)
Issues with urine protein methods

- Different proteins have different measurement responses with the same method
- A given protein has a different response in different methods
- Variable influence of interfering substances on different methods
- No standard reference material for calibration
Mean total protein of 12 urine samples measured by 7 methods, and using 3 standard materials

<table>
<thead>
<tr>
<th>Standard</th>
<th>SSA</th>
<th>SSA-SS</th>
<th>TCA</th>
<th>BC</th>
<th>CBB</th>
<th>PR-M</th>
<th>TCA-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA</td>
<td>1.80</td>
<td>2.44</td>
<td>4.71</td>
<td>2.75</td>
<td>2.59</td>
<td>2.93</td>
<td>3.14</td>
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<tr>
<td>HSA</td>
<td>1.25</td>
<td>3.71</td>
<td><strong>5.12</strong></td>
<td>2.90</td>
<td>2.75</td>
<td>2.59</td>
<td>2.99</td>
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<tr>
<td>Serum</td>
<td>3.39</td>
<td>3.26</td>
<td>3.98</td>
<td>2.78</td>
<td>2.75</td>
<td>2.95</td>
<td>2.86</td>
</tr>
</tbody>
</table>

**Patients:**
(3) nephrotic syndrome
(1) diabetic nephropathy
(1) systemic lupus
(1) acute glomerulonephritis
(2) multiple myeloma
(4) cancer

**Methods:**
SSA – sulfosalicylic acid
SSA-SS - sulfosalicylic acid sodium sulfate
TCA – trichloroacetic acid
BC – benzethonium chloride
CBB – comassie brilliant blue
PR-M – Pyrogallol red molybdenenum
TCA-B - Trichloroacetic acid precipitation biuret
Summary: measurement issues

• Creatinine calibration is standardized

• Influence of interfering substances is method dependent (for both Jaffe and enzymatic)

• Standardization of Cystatin C is underway

• Urine albumin methods are more robust and uniform than urine protein methods

• A reference system to standardize urine albumin is in development

• Urine protein is highly variable among methods