# Laboratory and Measurement Issues

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# Outline

- **Serum/plasma creatinine**
- Serum/plasma cystatin C
- Urine albumin
- Urine protein

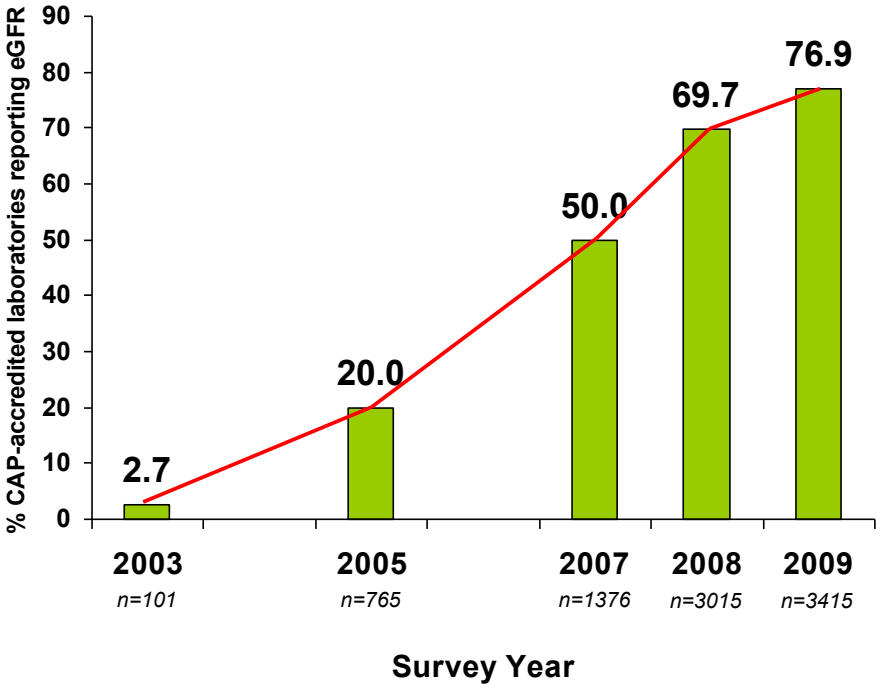
# Creatinine standardization

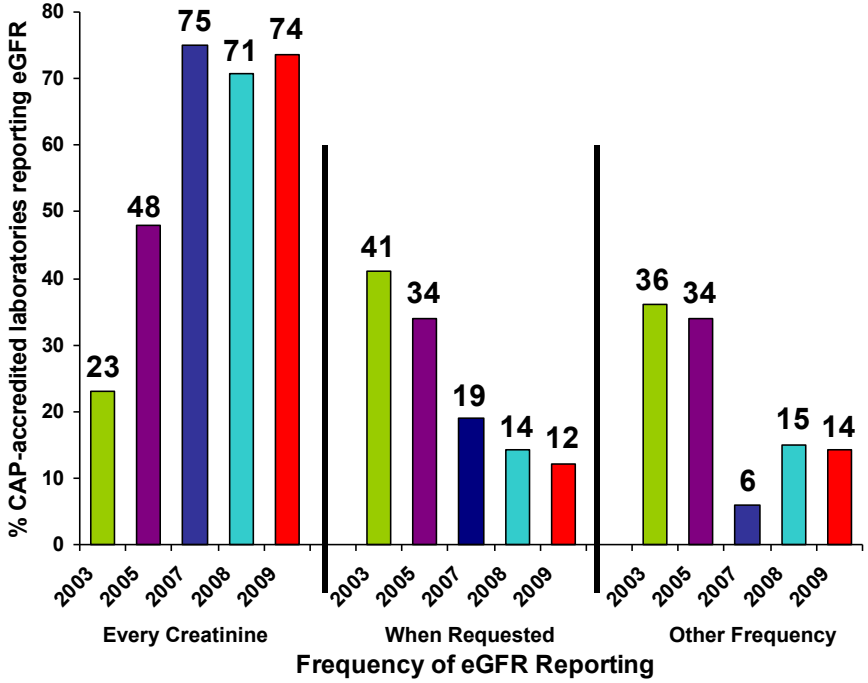
By mid 2010, all creatinine methods will have calibration traceable to isotope dilution mass spectrometry (IDMS) reference measurement procedures

- 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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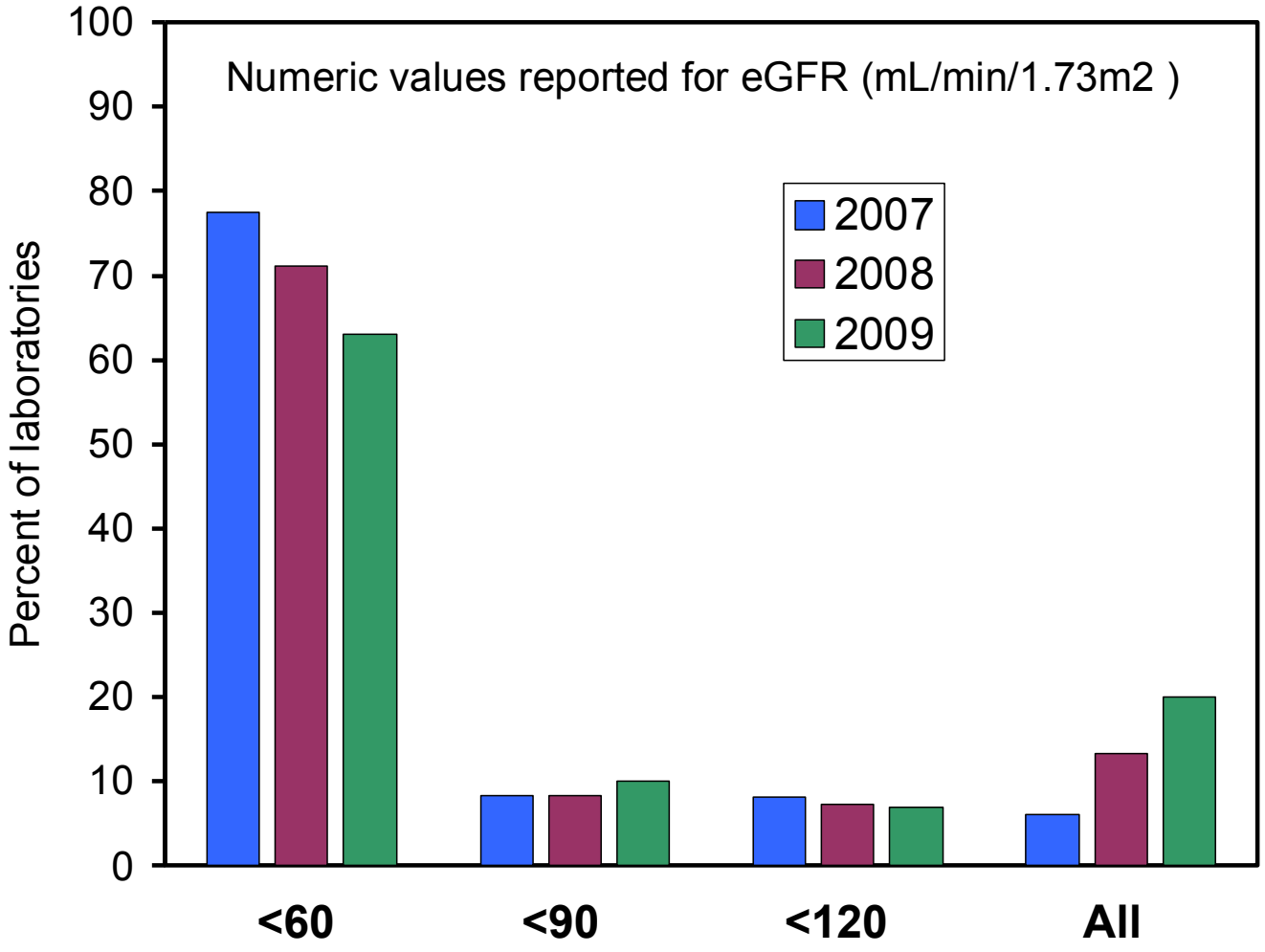

# Specificity of creatinine methods

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 Jaffe (alkaline picrate) methods are influenced by interfering substances
- Enzymatic methods have fewer interfering substance influences than Jaffe
- 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

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- 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

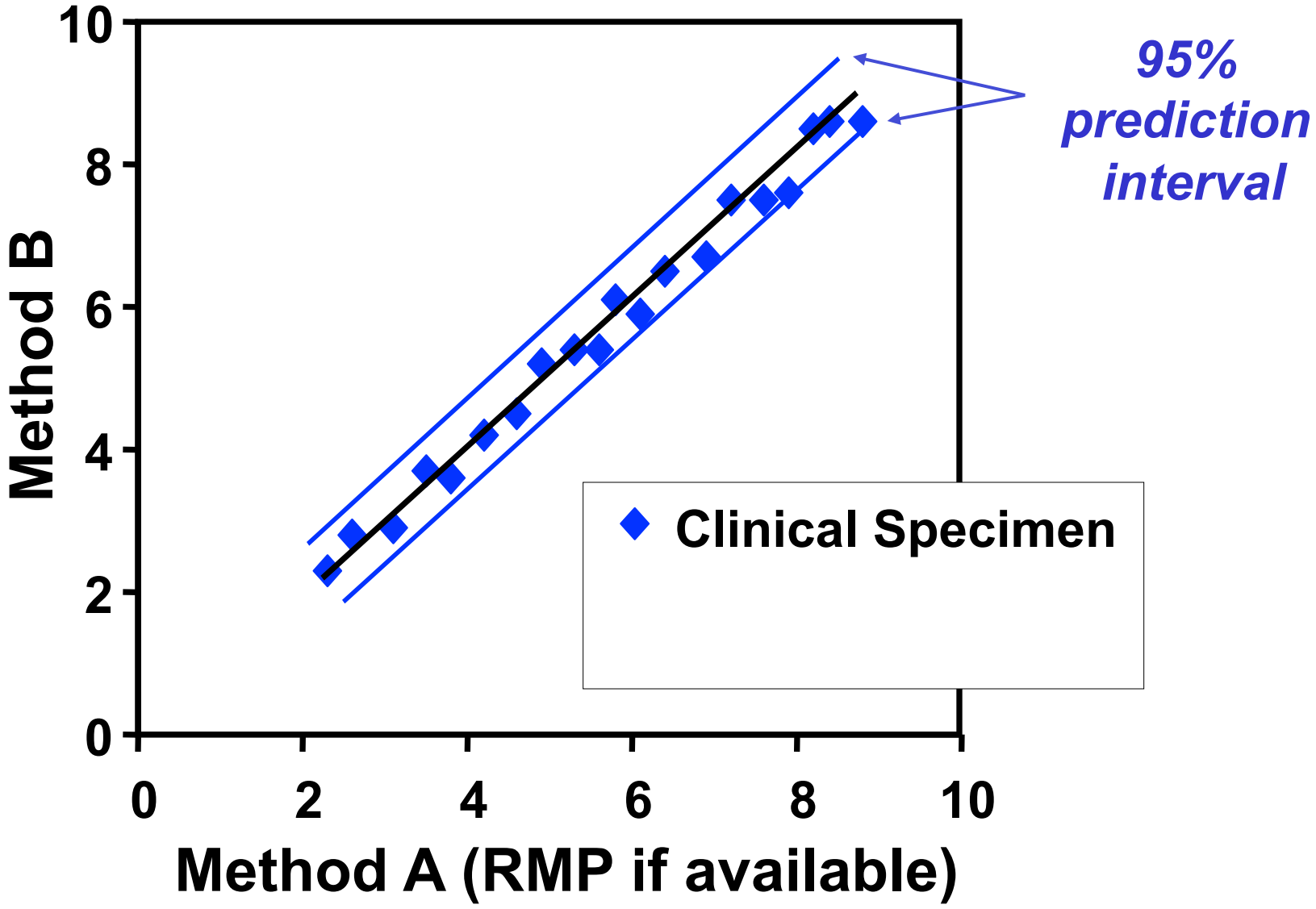
IFCC work group (chair: A. Grubb)

- 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

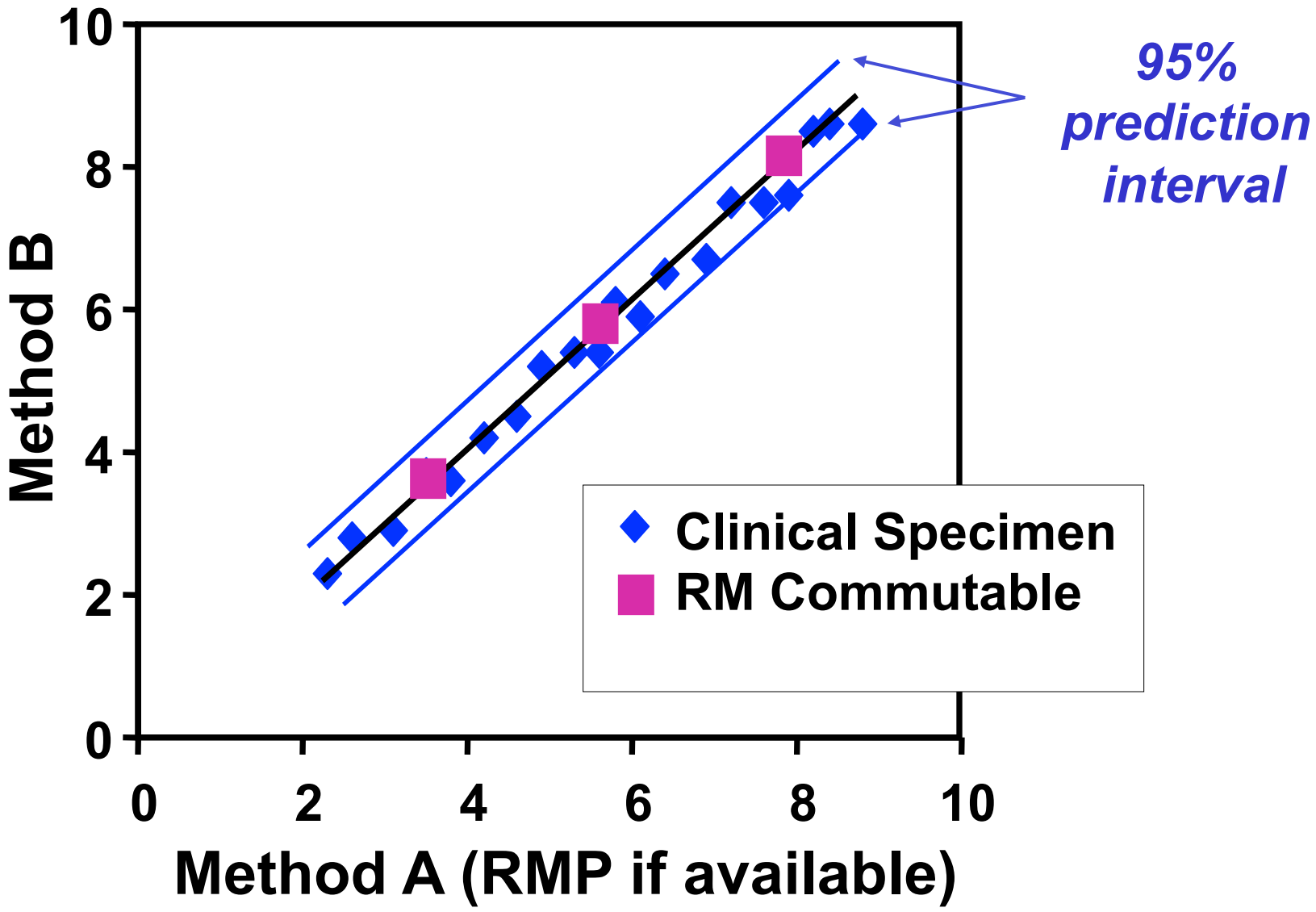
# Commutable reference material

- 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.

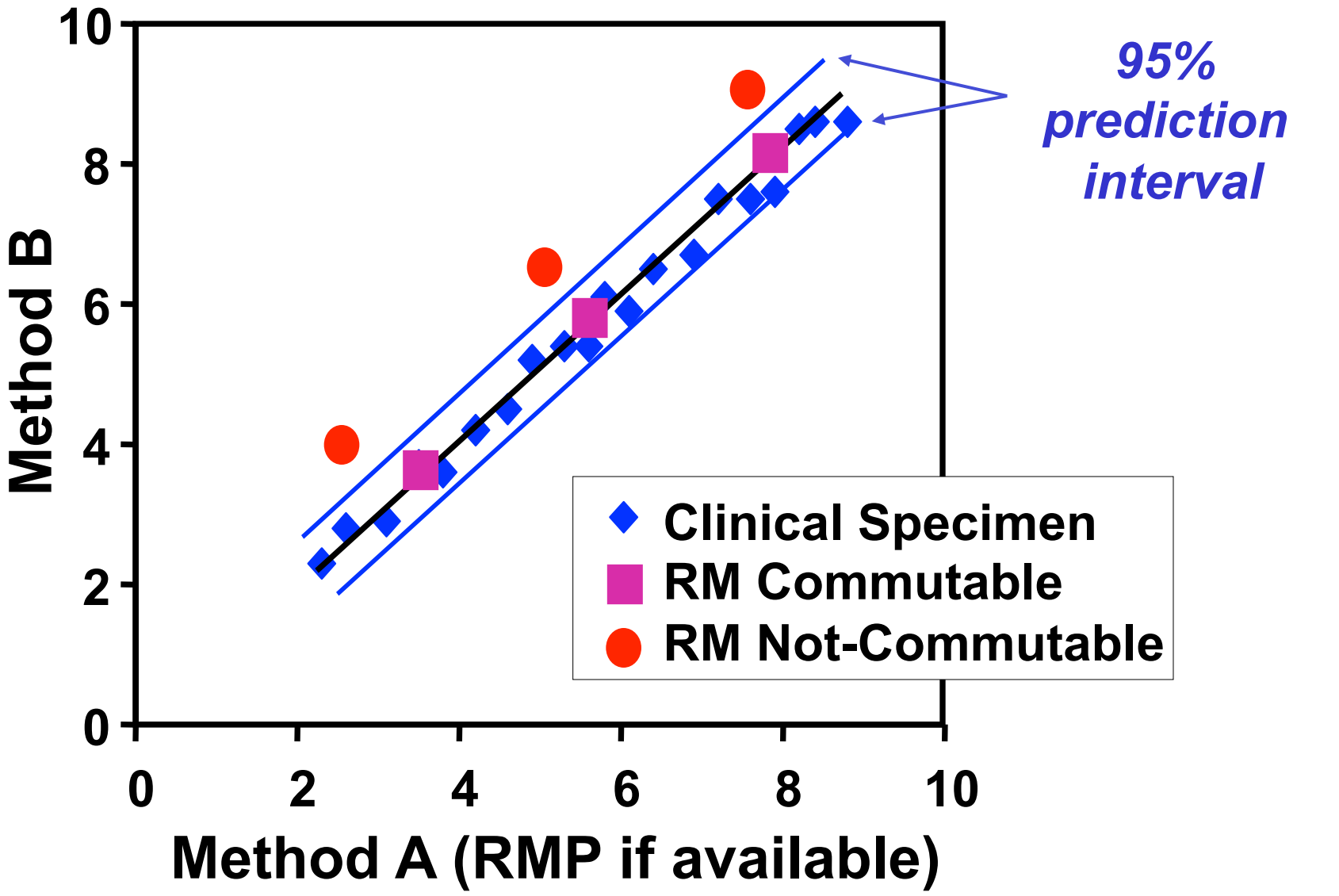
# Numeric relationship for patients



# Commutable if same as patients



# Not-commutable if different than patients



# 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
- Serum/plasma cystatin C
- **Urine albumin**
- Urine protein

# Standardization of urine albumin and creatinine measurement and reporting

NKDEP/IFCC conference held in March 2007

*Clinical Chemistry* 2009; 55: 24-38.

# Albumin in urine is heterogeneous

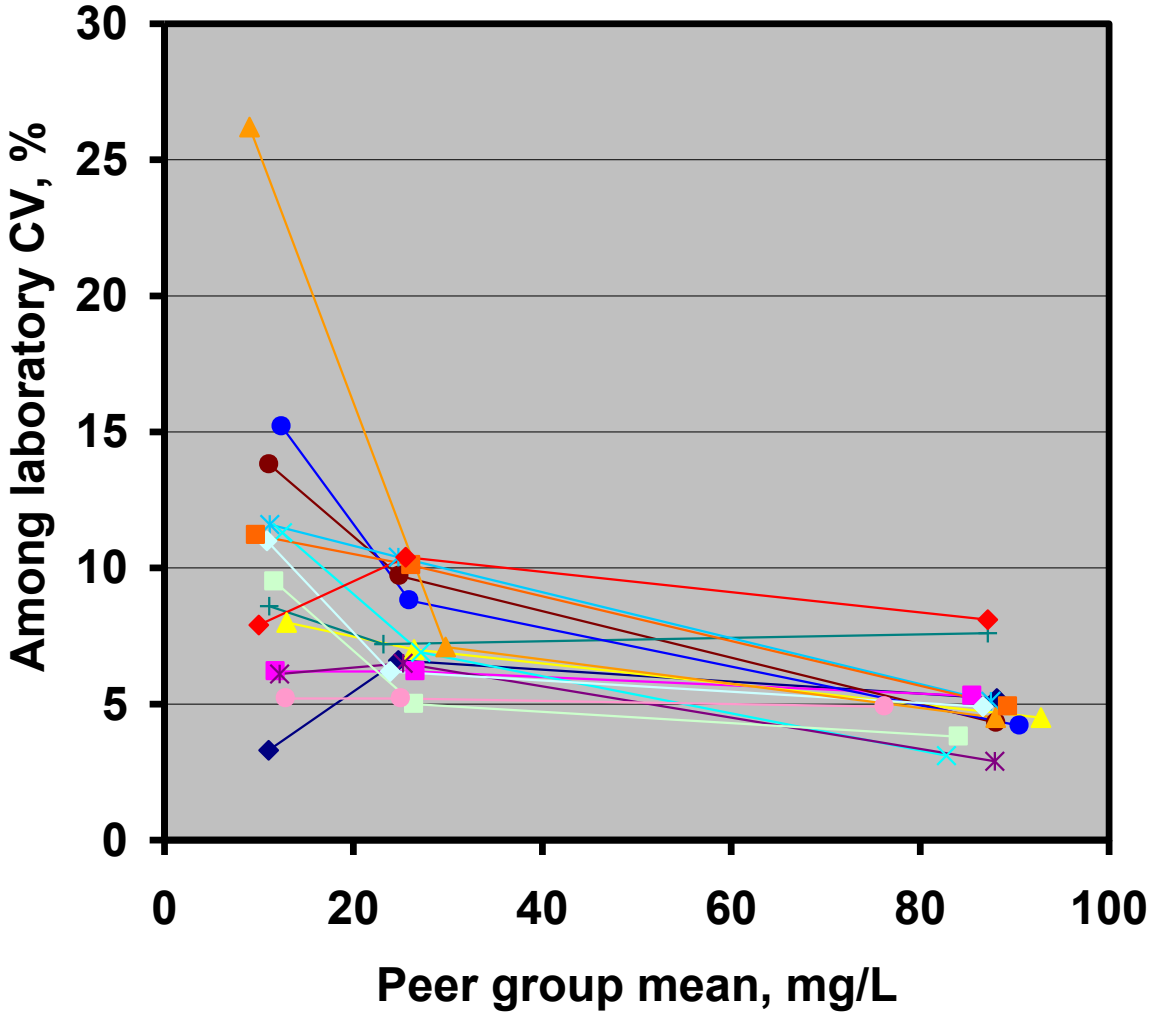
- Large and small fragments exist in plasma and urine
- C- and N-terminal truncation occurs
- Tubular uptake is receptor mediated – influences enrichment of modified plasma forms in urine (e.g. glycated)
- Many ligands are concentrated in urine and bind to albumin
- Proteolytic degradation and chemical 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

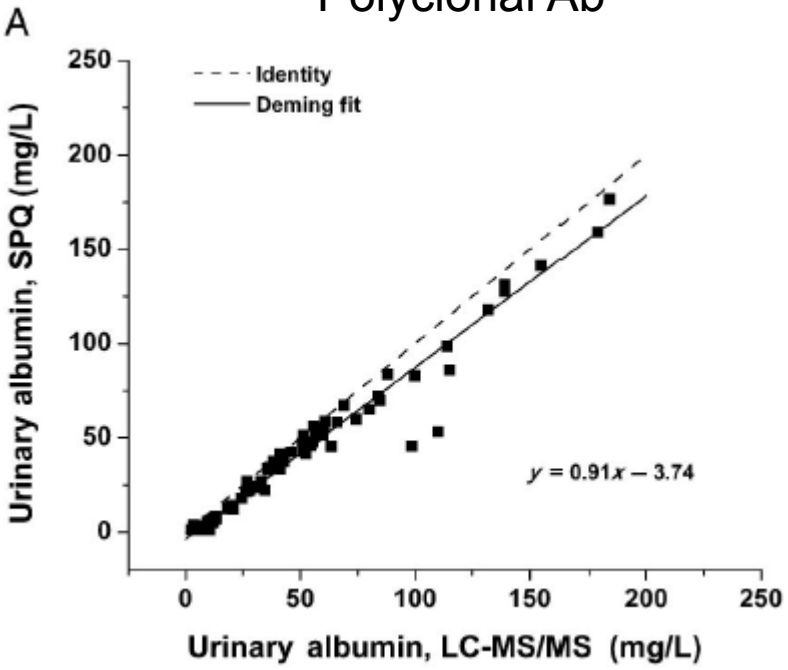


# Immunoassay vs LC-MS

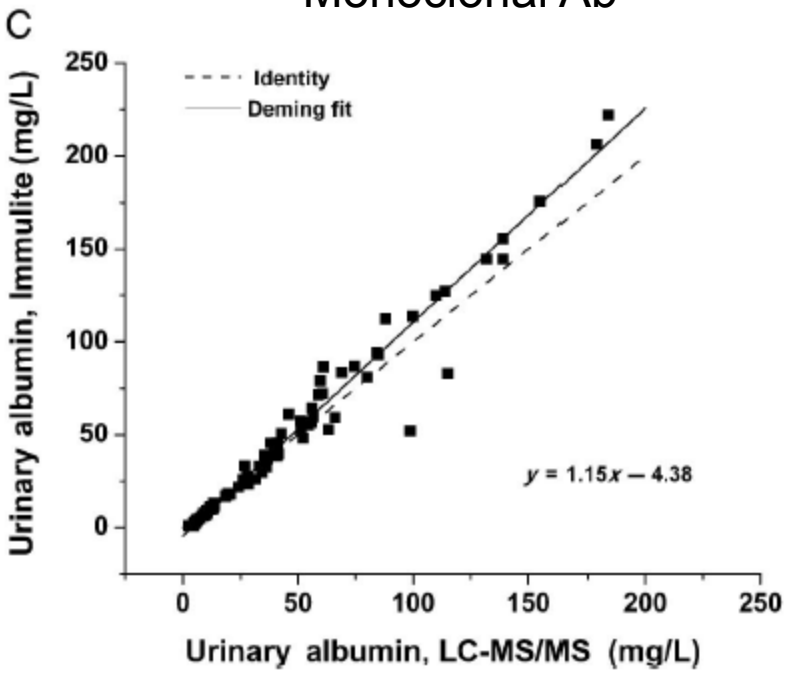
Average difference = 24%

(N = 92 patient urines)

Polyclonal Ab




Monoclonal Ab



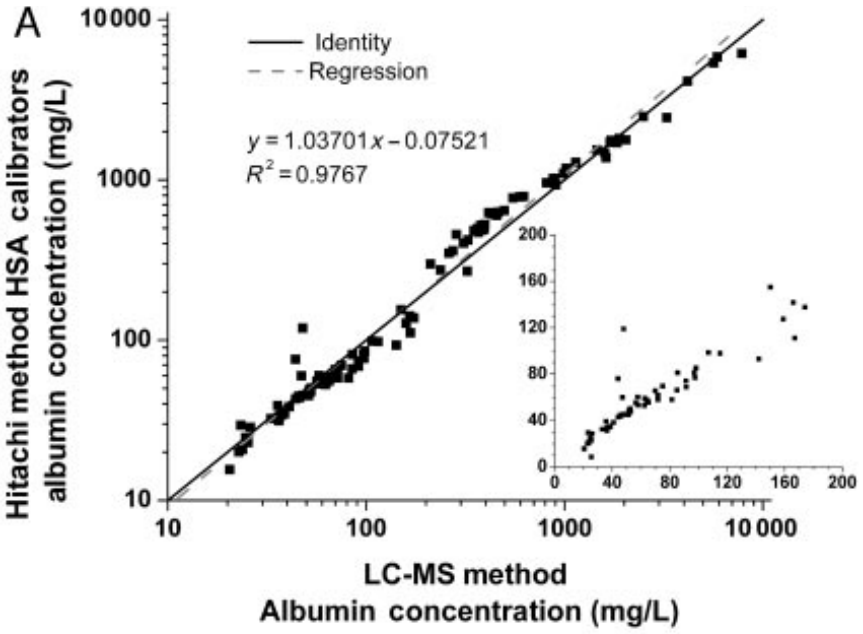

Seegmiller et al. Clin Chem 2009;55: epub

# Albumin measurement procedures

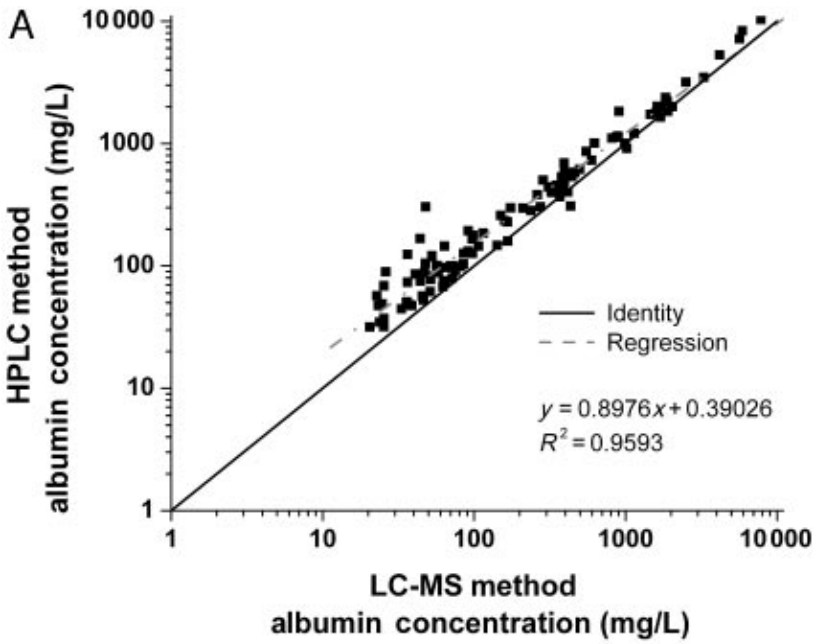
- HPLC assays (size exclusion)
  - Does not resolve albumin from other co-eluting urine proteins causing overestimation
  - Hypothesis of “non-immunoreactive albumin” likely related to non-specificity of HPLC

# Immunoassay and HPLC vs LC-MS

Immunoassay




HPLC




Shaikh et al. Clin Chem 2008; 54: 1504-1510

# Immunoassay and HPLC for predicting cardiovascular events

| | Areas under ROC Curves | |
|---|---|---|
| | Immunoassay | HPLC |
| All Participants (N = 5,358) | 0.612 (0.586 - 0.638) | 0.581 (0.535 - 0.609) |
| With Diabetes (N =1,992) | 0.593 | 0.564 |
| Without Diabetes (N = 3366) | 0.612 | 0.574 |

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
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- **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

| Standard | Mean total protein, g/L (n = 12) | | | | | | |
|---|---|---|---|---|---|---|---|
| | SSA | SSA-SS | TCA | BC | CBB | PR-M | TCA-B |
| BSA | 1.80 | 2.44 | 4.71 | 2.75 | 2.59 | 2.93 | 3.14 |
| HSA | 1.25 | 3.71 | 5.12 | 2.90 | 2.75 | 2.59 | 2.99 |
| Serum | 3.39 | 3.26 | 3.98 | 2.78 | 2.75 | 2.95 | 2.86 |

**Patients:**
(3) nephrotic syndrome
(1) diabetic nephropathy
(1) systemic lupus
(1) acute glomerulonephritis
(2) multiple meyoloma
(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 molybdenum
TCA-B - Trichloroacetic acid precipitation biuret

Imai, Clin Chem 1995;32,1986.

# 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