Standardization of Creatinine: Finally Achieved?

Presented at the 7th CIRME International Scientific Meeting
Metrological traceability and assay standardization
Stresa, Italy

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Washington, DC

The National Kidney Disease Education Program (NKDEP)

In 2000 the US National Institutes of Health created the National Kidney Disease Education program (NKDEP) to address the epidemic of kidney disease in the US.

The NKDEP Laboratory Working Group (LWG) was created to review and address the problems related to serum creatinine measurement for estimating GFR, and to prepare recommendations to standardize and improve creatinine measurement.

MDRD equation for estimating GFR in adults age 18 and older

GFR (mL/min/1.73 m^2) =

175* x Creatinine (serum)$^{-1.154}$

x Age$^{-0.203}$

x 0.742 (If Female)

x 1.210 (If African-American)

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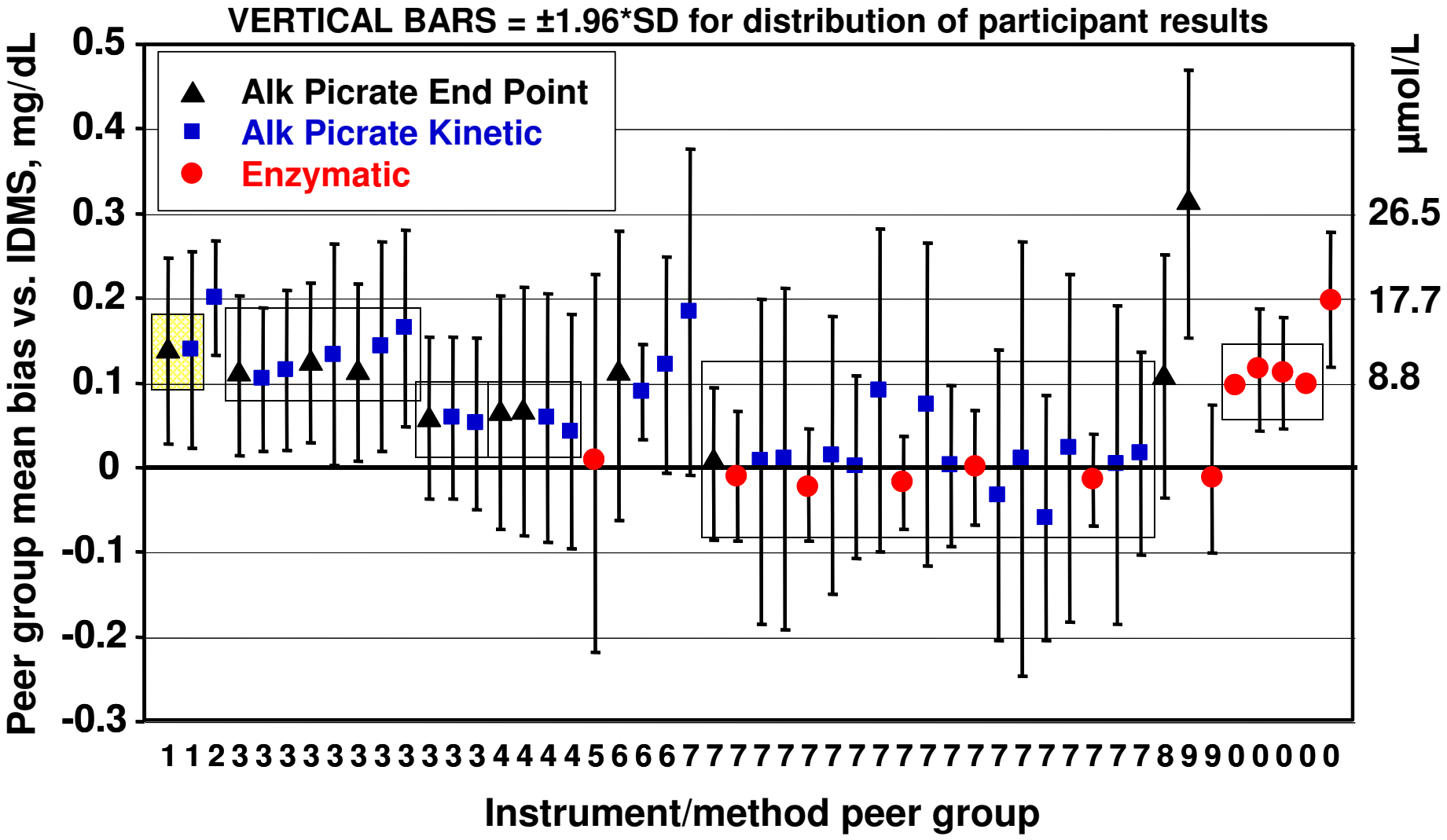
* 175 for calibration traceable to IDMS

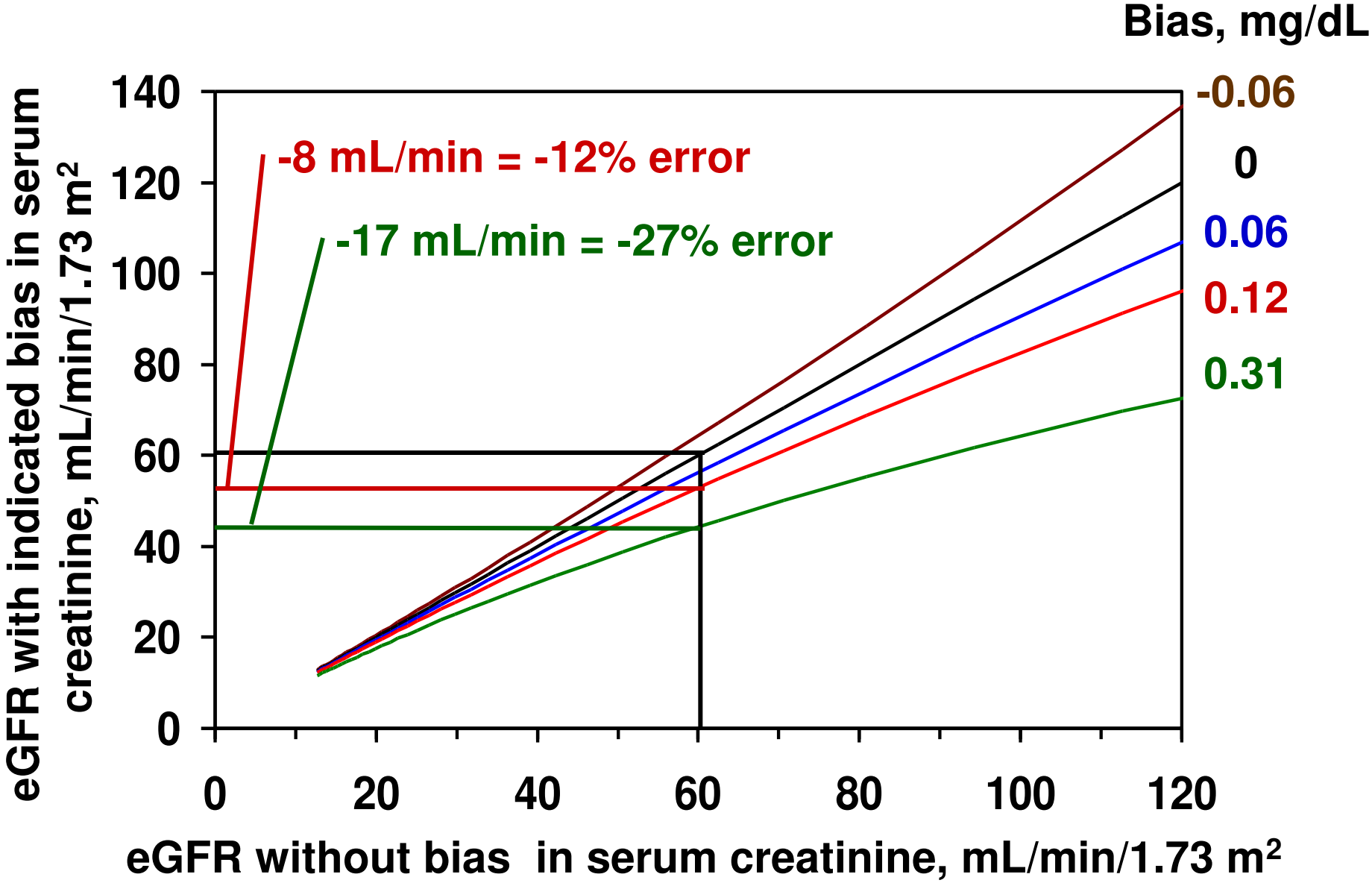
* use 186 for traditional calibration;

CAP, 2003, Fresh Frozen Serum, N = 5624
Creatinine = 0.90 mg/dL (79.7 µmol/L)



Miller et al. Arch Pathol Lab Med 2005;129:297-304

Impact of creatinine bias on GFR



Myers et al. Clin Chem 2006;52:5-18

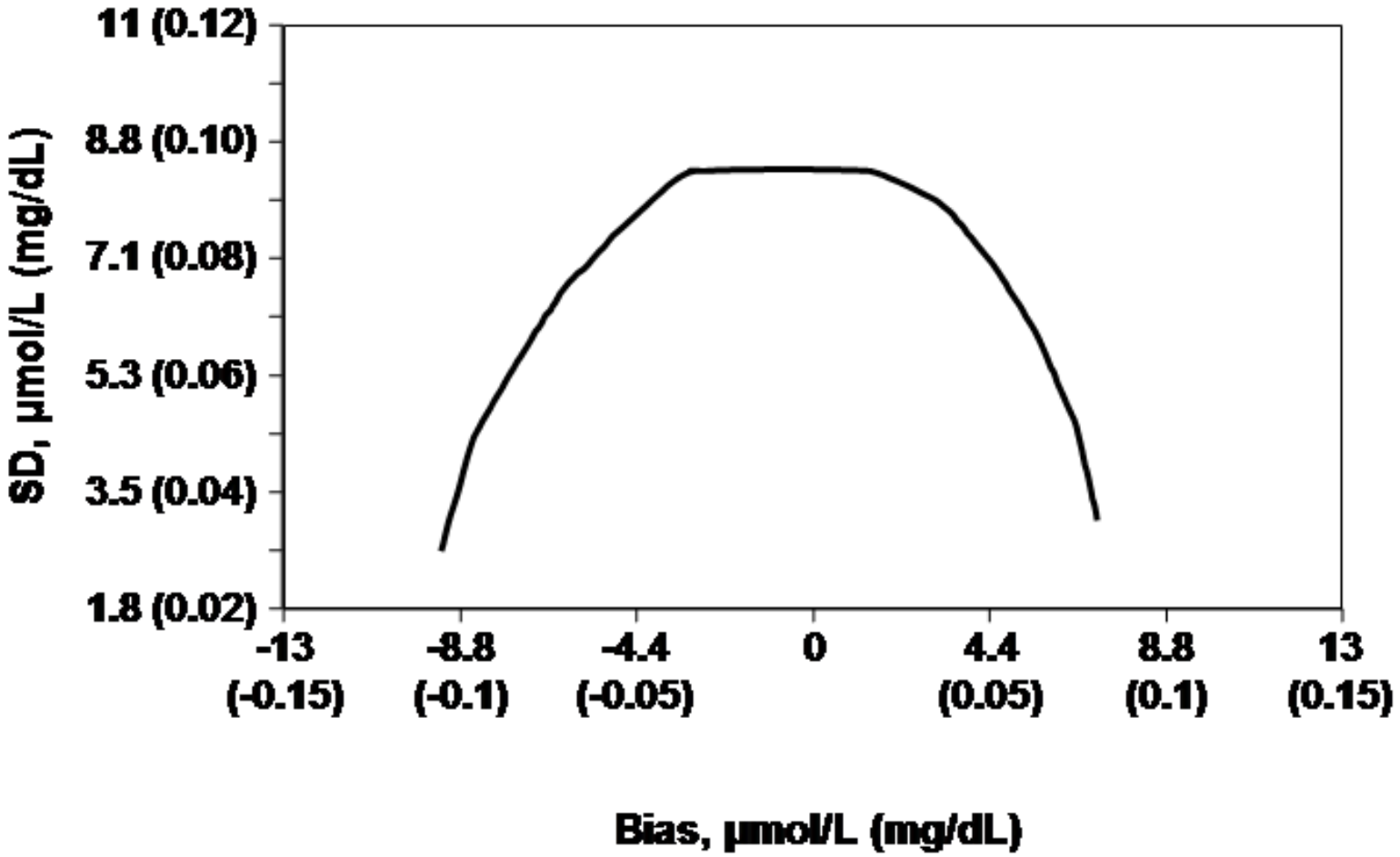
Impact of creatinine method performance on estimated GFR

- As creatinine values go lower (GFR is higher/less CKD) measurement bias and imprecision have a larger impact on eGFR variability
  - These measurement limitations are part of the reason NKDEP recommends not to report eGFR values >60 mL/min/1.73m^2
  - At GFR <60 mL/min/1.73m2 (higher creatinine) the bias and imprecision have less impact on the variability, and thus the clinical reliability of eGFR

Recommended creatinine method performance needed

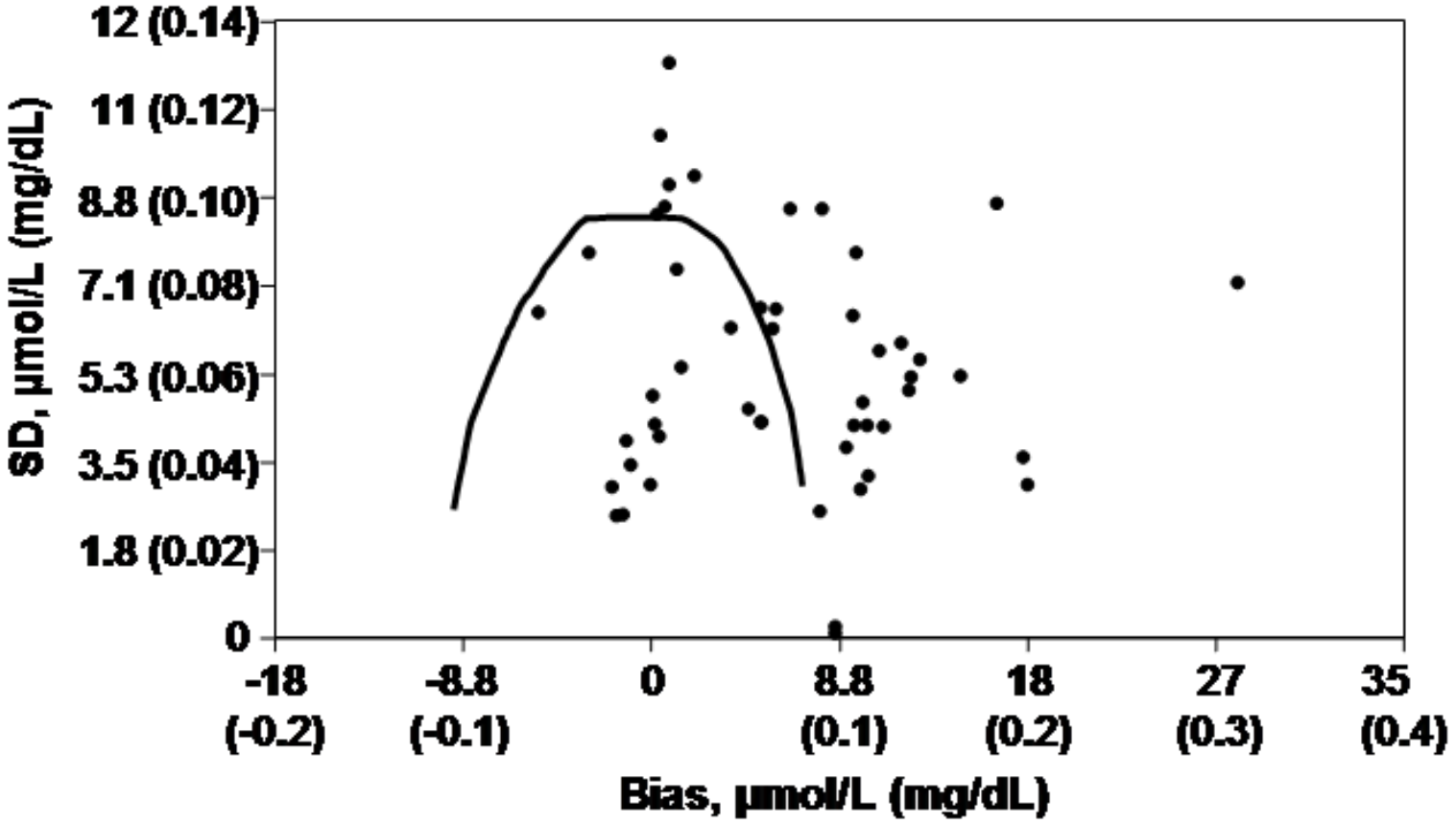
- Total error in creatinine measurement should not increase the variability in eGFR more than 10% in the critical creatinine range 1.0-1.5 mg/dL (88-133 µmol/L)
- Recommended method performance that would ensure a <10% increase in the variability of eGFR at a serum creatinine concentration of 1.0 mg/dL (88 µmol/L)
  - Imprecision: Standard Deviation <0.08 mg/dL (7.1 µmol/L)
  - Bias: <0.05 mg/dL (4.4 µmol/L) compared to an IDMS reference measurement procedure.

Total Error budget for creatinine measurement in the range 1.00-1.50 mg/dL



Myers et al. Clin Chem 2006;52:5-18

Performance of routine methods compared to the TE limit for creatinine



Myers et al. Clin Chem 2006;52:5-18

Creatinine calibration standardization program

- Eliminate the bias between different methods
  - Make calibration traceable to IDMS reference measurement procedure (gold standard)
- Improve the accuracy and consistency of estimated GFR

Tools for creatinine measurement standardization

- US National Institute of Standards and Technology
  - ID-GC/MS & ID-LC/MS Reference Measurement Procedures
- NIST Standard Reference Material (SRM 967a)
  - Fresh-frozen human serum pools prepared according to CLSI C-37A
  - Two levels:
    - **0.847 mg/dL (74.9 µmol/L)**
    - **3.88 mg/dL (343 µmol/L)**

Tools for creatinine measurement standardization

- CAP LN24 Creatinine Accuracy/Linearity Survey
  - Designed to provide an accuracy-based assessment of a clinical lab's serum creatinine measurements in the normal and slightly elevated range
  - Six specimens provided to labs
  - Three specimens value-assigned by RMP
  - Levels for remaining specimens computed based on admixture ratios established during preparation

Commutability Study for Creatinine Materials

- In 2006 a commutability study was performed using CLSI EP14-A2: *Evaluation of Matrix Effects*
- The following vendors participated
  - Abbott Diagnostics
  - Beckman Coulter
  - Dade Behring
  - Olympus
  - Roche
  - Ortho Diagnostics
  - Siemens
- SRM 967 and LN24 pools demonstrated commutability with the methods evaluated

JCTLM Listing of RMPs for Creatinine

Reference Measurement Procedures	
ID/GC/MS	3
ID/LC/MS	3

Reference Laboratory Services		
Laboratory	Country	RMP
DGKL	Germany	ID/GC/MS
INSTAND e.V.	Germany	ID/GC/MS
LNE	France	ID/GC/MS
UGent	Belgium	ID/GC/MS
WEQAS	UK	ID/GC/MS

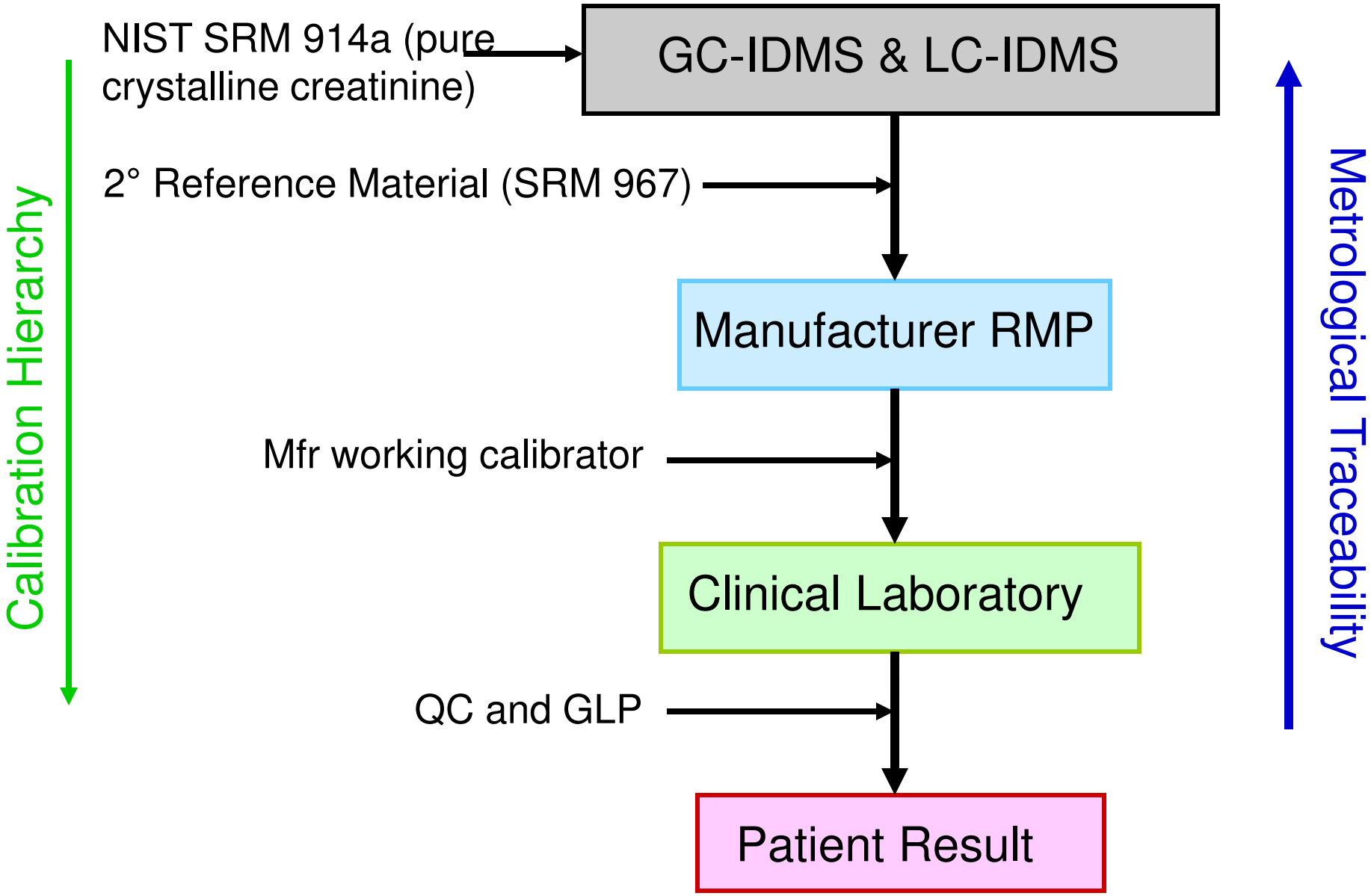
www.bipm.org/jctlm accessed 05/13/2013

JCTLM Reference Material Listing for Creatinine in Serum

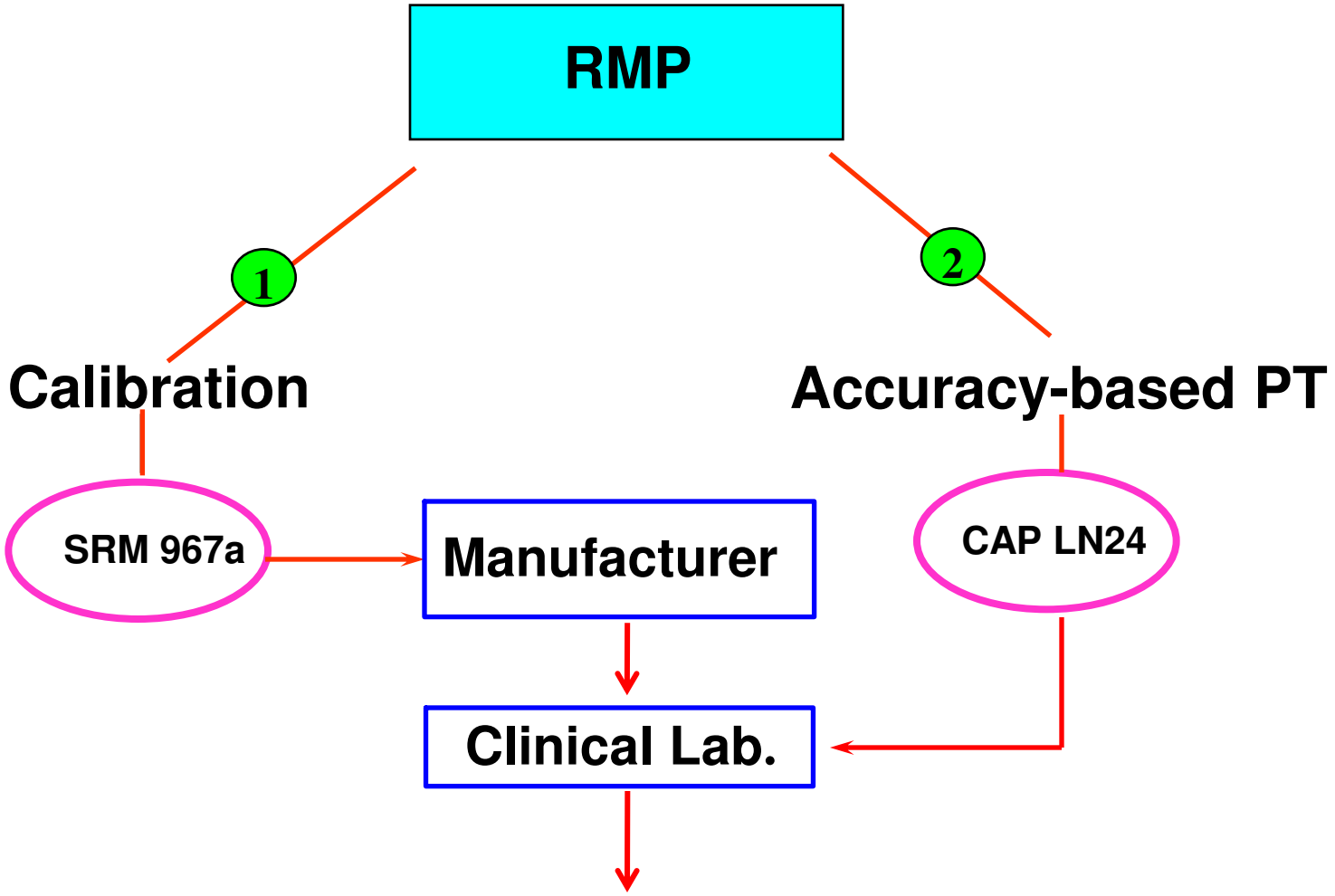
Organization	Material	Assigned Value
Centro Nacional de Metrologia (CENAM), Mexico	DMR 263a	0.0664 mmol/L
NIST	SRM 967a	Level 1 – 0.0749 mmol/L
		Level 2 – 0.3427 mmol/L
IRMM	BCR 573	68.7 µmol/L
	BCR 574	105 µmol/L
	BCR 575	404.1 µmol/L
LGC Limited, UK	ERM DA250a	39 mg/kg
	ERM DA251a	22 mg/kg
	ERM DA252a	3.1 mg/kg
	ERM DA253a	50 mg/kg
All materials listed in compliance with ISO 15194:2003/ not 15194:2009		

www.bipm.org/jctlm accessed 05/13/2013

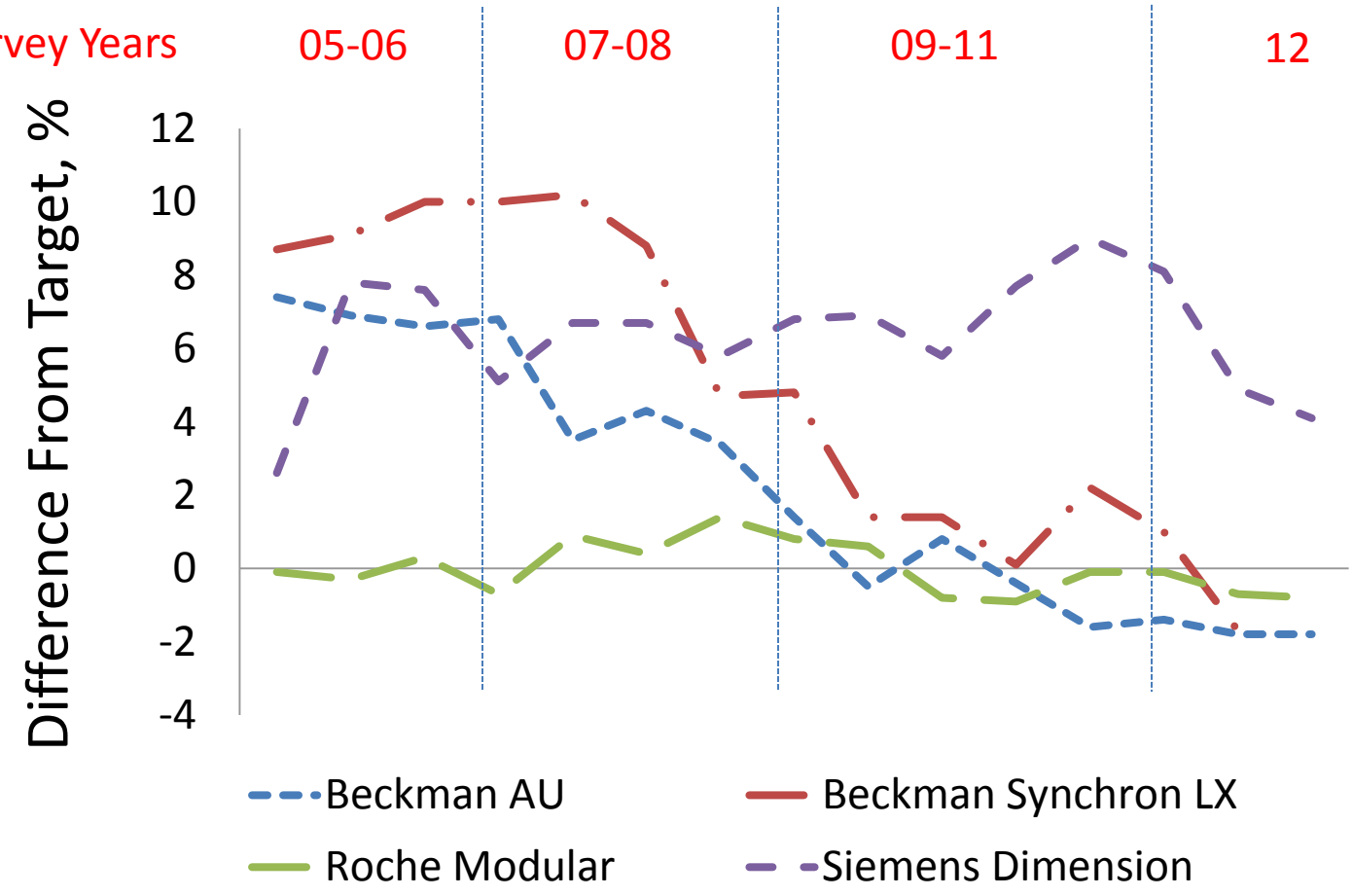
Traceability Chain for Creatinine Measurement



Creatinine Standardization



Percent Bias by Instrument/Mailing for CAP LN24 Survey



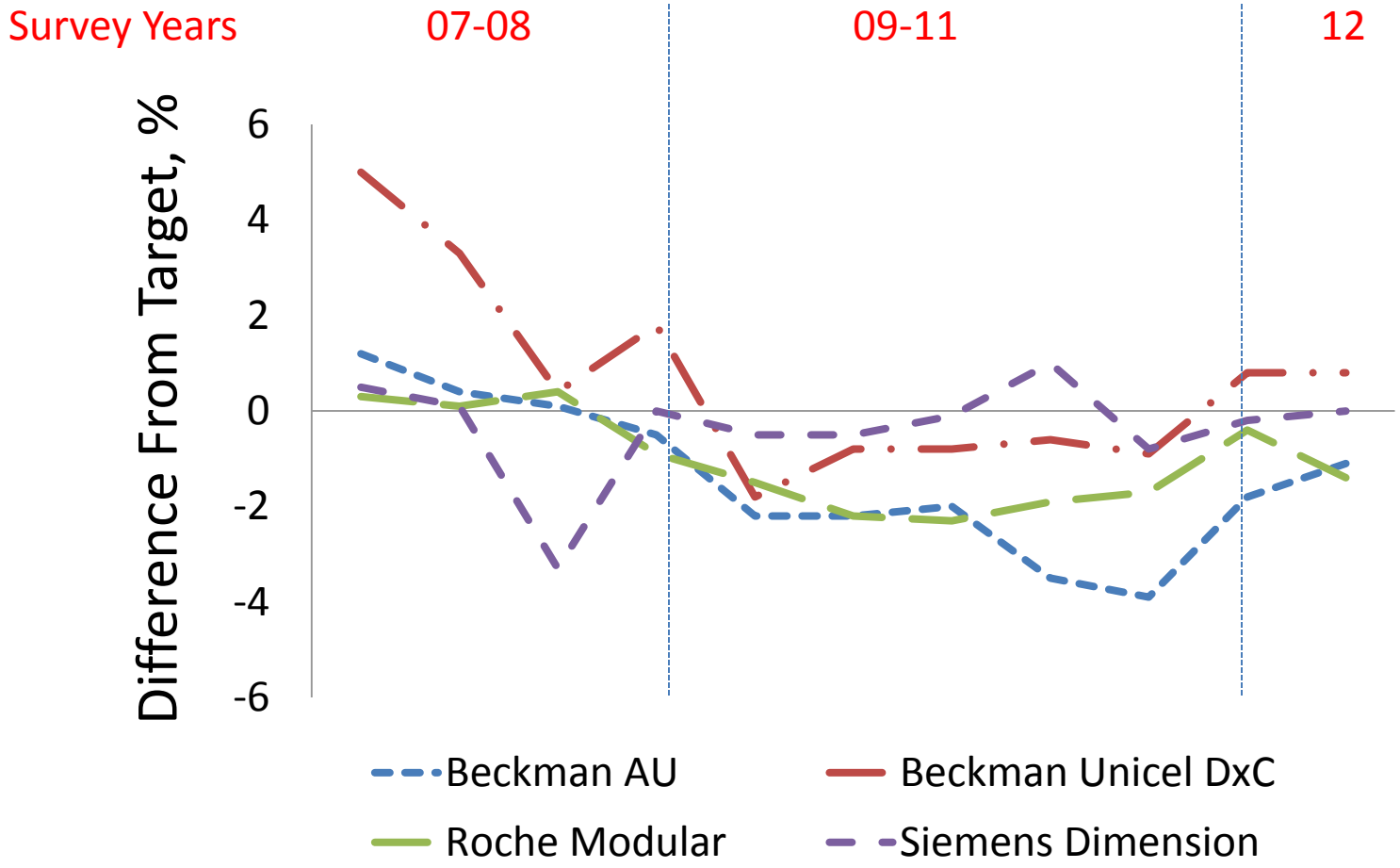
05-06 target = 0.739 mg/dL 07-08 target = 0.794 mg/dL

09-11 target = 0.770 mg/dL 12 target = 0.707 mg/dL

Results from CAP LN24 2012A/B used with permission

Arch Pathol Lab Med 2013;137:496.

Percent Bias by Instrument/Mailing for CAP LN24 Survey

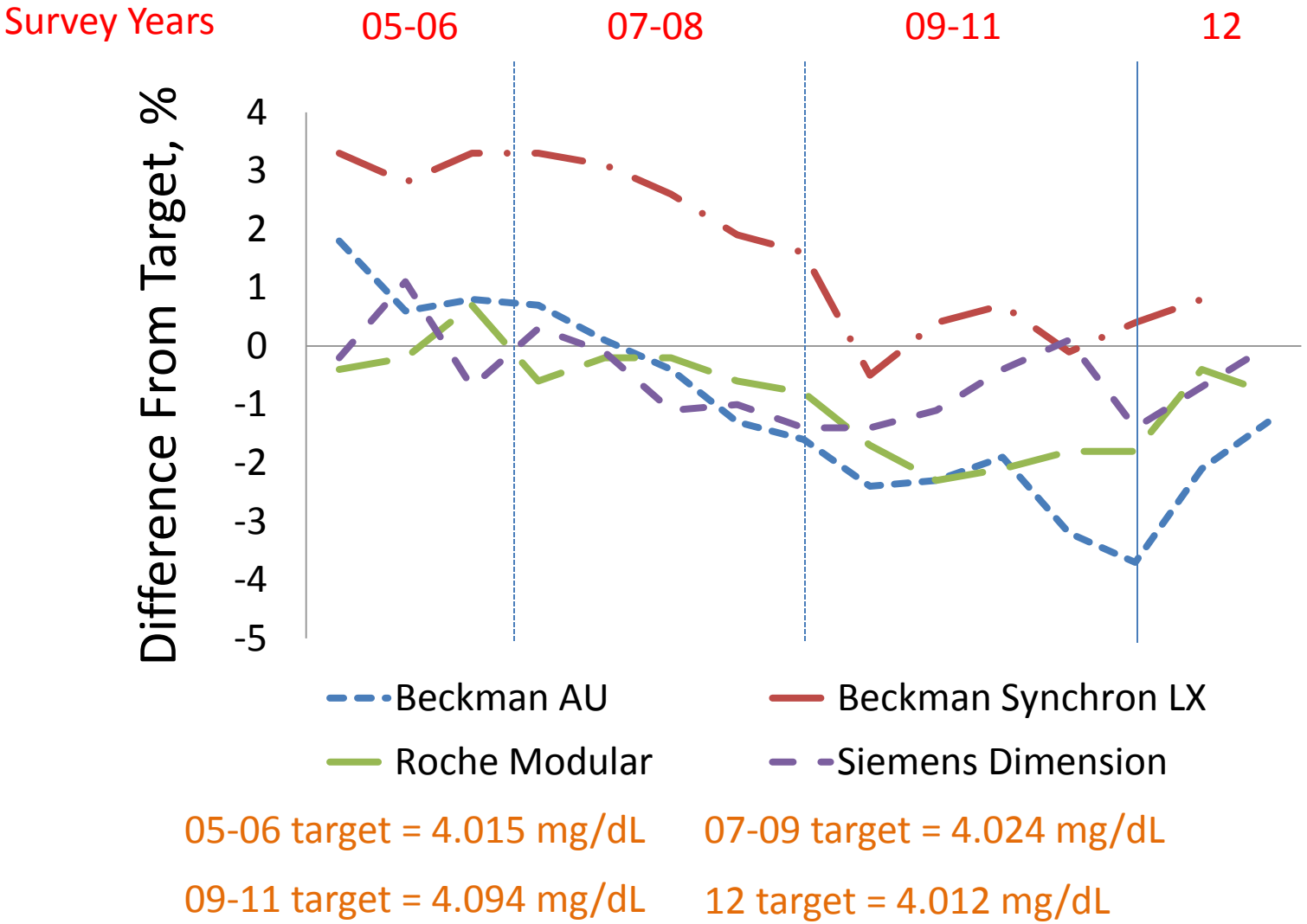


07-08 target = 2.727 mg/dL 09-11 target = 2.766 mg/dL 12 target = 2.692 mg/dL

Results from CAP LN24 2012A/B used with permission

Arch Pathol Lab Med 2013;137:496

Percent Bias by Instrument/Mailing for CAP LN 24 Survey



Results from CAP LN24 2012A/B used with permission

Arch Pathol Lab Med 2013;137:496

Summary Comparison of Creatinine Performance

Instrument	Method	2005*		2011**		2012**	
		Mean	Bias, %	Mean	Bias, %	Mean	Bias, %
IDMS Target (mg/dL)		0.902		0.770		0.707	
Abbott (Aeroset) Arch C	AP-K	1.04	15.5	0.75	-2.6	0.69	-2.4
Beckman Synchron LX	AP-K	0.96	6.6	0.77	0	0.69	-2.4
Beckman (Olympus) AU	AP-K	0.99	10.0	0.76	-1.3	0.69	-2.4
Roche Modular	AP-RB	0.92	1.1	0.78	1.3	0.72	1.8
Roche Modular	E	0.90	0	0.76	-1.3	0.69	-2.4
Siemens (Bayer)Advia	(AP-K) AP-RB	1.10	22.2	0.76	-3.9	0.71	0.4
Vitros (950) 5.1 FS	E	1.00	11.1	0.76	-1.3	0.69	-2.4

Methods included AP-K, alkaline picrate kinetic; AP-RB, alkaline picrate rate-blanked compensated kinetic; E, enzymatic

* Arch Pathol Lab Med 2005;129(3):297.

** Results from CAP LN24 2011A and LN24 2012A used with permission.

Creatinine Results from SKML EQAS

Method	N	Mean, µmol/L	SD, µmol/L	Bias, %
Alkaline picrate kinetic	99	66.0	5.7	1.8
Enzymatic	126	65.8	3.0	1.5

Target mean = 64.8 µmol/L; Assigned by IDMS at DGKL, Germany

Sample type – Fresh frozen serum spiked with creatinine

Results used with permission

Percentage of Failures in CEQAL PT Program

Method Group		Creatinine IDMS Assigned Value		
		Sample A	Sample B	Sample C
	n	< 80 µmol/L	112.9 µmol/L	139.2 µmol/L
Alkaline picrate	23	59%	15%	6%
(Not IDMS traceable)				
Alkaline picrate (blanked)	36	74%	24%	27%
(IDMS traceable)				
Alkaline picrate	91	39%	8%	9%
(IDMS traceable)				
Creatininase (amidohydrolase)	103	41%	13%	9%
(IDMS traceable)				

Desirable TE goal = 7.6% relative to IDMS assigned value

Clinical Biochemistry 2012;45:279.

EQAS of the Argentine Biochemical Foundation

- 3300 registered clinical laboratories (gov & private)
  - 11% use homogeneous analytical systems
  - 89% use heterogeneous analytical systems

Creatinine Performance Evaluation

Participant Parameters

- Laboratories: 39 clinical labs (public & private)
- Instruments: Automated (17); Semi-automated (15); Manual (7)
- Assay Type: Jaffe EP (5); Jaffe Kinetic (31); 3 no Info
- Calibration: Serum-based (18); Aqueous (7); 14 no info
- Homogeneous systems (5)
- Heterogeneous systems (34)

Creatinine Performance Evaluation

Study Protocol

- Secondary Reference Measurement Procedure
  - Roche enzymatic
  - Calibrated with NIST SRM 967
  - Validated by participation in IFCC RELA
- Serum panels at 4 different levels
- Value assigned over 3 days, independent calibration, 4 replicates /level
- Clinical labs analyzed panel on each of 3 days in triplicate

Creatinine Performance Evaluation

Study Results

Parameters	Level 1	Level 2	Level 3	Level 4
Reference Value (mg/dL)	0.65	1.01	1.59	2.28
Lab Values (mg/dL)	1.02	1.39	1.96	2.64
CV (%)	18.9	15.2	14.7	14.0
n	347	348	351	351
Diff from RMP (%)	+57	+38	+23	+16

Global Creatinine Assay Providers

Company	Assay Type	Calibration Reported	Country
Accurex	Jaffe	Not specified	India
AMS Diagnostics	Jaffe	Aqueous or serum	USA
Arkray	Reagent Strip	Spot chem	Japan
Audit Diagnostics	Jaffe	Standard (177µmol/L)	Ireland
BioMed Diagnostics	Jaffe, colorimetric EP	Standard (2.0 mg/dL)	Egypt
BioMed Diagnostics	Jaffe, fixed rate	Standard (2.0 mg/dL)	Egypt
Biotecnia	Jaffe	Traceable to NIST 914a	Brazil
BQ Kits	Enzymatic	Traceable to NIST 914a	USA
Chemhouse	Jaffe	Standard (2.0 mg/dL)	Pakistan
Diasys	Jaffe	Standard (2.0 mg/dL)	Germany

Personal communication from Dr. Graham Jones

Global Creatinine Assay Provides –cont'd

Company	Assay Type	Calibration Reported	Country
Diasys	Enzymatic	Standard (2.0 mg/dL)	Germany
Fortress Diagnostics	Jaffe	Aqueous or serum	UK
Fortress Diagnostics	Jaffe, deproteinized	Standard (177µmol/L)	UK
Fortress Diagnostics	Jaffe, deproteinized	Aqueous/Serum	UK
Fujifilm	Enzymatic	Traceable to NIST 914a	Japan
Genzyme	Jaffe	Not supplied	Canada, UK
ISESrl	Jaffe	Multicalibrator	Italy
IBL America	Enzymatic	Traceable to NIST 914a	USA
Piccolo	Enzymatic	Fullers earth as RMP	USA
Pointe Scientific	Jaffe	Standard (2.5 mg/dL)	USA

Personal communication from Dr. Graham Jones

Global Creatinine Assay Providers – cont'd

Company	Assay Type	Calibration Reported	Country
Robonik	Jaffe	Standard (2.0 mg/dL)	India
Sentinel	Jaffe	No information	Italy
Sentinel	Enzymatic	No information	Italy
Spinreact	Jaffe	Serum recommended	Spain
Spinreact	Enzymatic	Serum recommended	Spain
Stanbio	Jaffe		
Thermo	Jaffe	Serum recommended	USA/UK
Thermo	Enzymatic	Serum recommended	USA/UK
VITROSCIENT	Jaffe	Standard mot defined	Egypt
Wiener	Jaffe	Standard (20 mg/L)	Argentina

Personal communication from Dr. Graham Jones

The Good News

- A well defined reference system for creatinine exists
- Calibration is now traceable to IDMS by all major IVD providers of creatinine assays
- Creatinine measurement has improved

The Bad News

- Many heterogeneous assay systems for creatinine measurement are in use around the world that are not traceable to the RMP
- Global standardization of creatinine measurement is not complete!

Thank You!

Evaluation of eGFR Calculations

CAP LN24-01 (0.707 mg/dL)			
Equation	Calibration Type	Acceptable	Unacceptable
MDRD	IDMS	176	3
	Traditional	36	2
CKD-EPI	IDMS	12	7
CAP LN24-03 (2.030 mg/dL)			
Equation	Calibration Type	Acceptable	Unacceptable
MDRD	IDMS	201	25
	Traditional	34	15
CKD-EPI	IDMS	16	1

LN24-01 was reported to be from an 18-year-old non-African American female.

LN24-03 was reported to be from a 50 year-old African American male.

Results from CAP LN24 2012 A/B used with permission

Creatinine methods

- Calibration is now standardized to IDMS by all major global manufacturers

However, standardization does not correct for non-specificity problems

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

- Drugs
- Endogenous substances, e.g.
  - Glucose
  - Ketoacidosis
  - Bilirubin
  - Hemoglobin
  - Protein

NKDEP – IFCC specificity evaluation

- 19 panels each with ~20 individual sera selected to include a wide range of potentially interfering substances
- 20 apparently healthy controls
- 4 enzymatic and 3 Jaffe creatinine methods
- Bias vs. ID-LC-MS/MS
- Trueness verified with NIST SRM 967

NKDEP – IFCC specificity evaluation

	Number of samples per group with a negative or positive bias >0.10 mg/dL (>8.8 mmol/L) or >10% whichever is greater														
	Highlight →	3-5 samples with bias					≥6 samples with bias								
		E1		E2		E3		E4		J1		J2		J3	
Subject group	n	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos
Apparently healthy subjects	20	0	1	0	0	0	0	0	0	0	0	1	0	0	1
Diabetes mellitus															
β-hydroxybutyrate, 33-103 mg/dL	19	0	0	0	3	0	1	0	0	0	11	5	1	0	14
Glucose, 388-816 mg/dL	20	1	2	0	1	1	0	0	0	0	14	0	8	0	19
HbA_{1c}, 8.1-13.2%	20	0	0	0	0	0	0	1	0	0	1	0	0	0	16
Cardiovascular disease w HTN	20	0	0	0	0	0	0	0	0	0	0	1	0	0	6
Drugs															
Cephalosporins	20	0	0	0	0	0	0	1	0	0	5	1	0	0	10
Dobutamine	18	3	0	1	0	2	0	2	0	0	3	0	0	0	7
Dopamine	11	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Lidocaine	20	0	10	0	11	0	9	0	8	0	4	4	0	0	5
Endogenous substances															
Bilirubin, 9-38 mg/dL	20	13	0	1	0	10	0	18	0	0	0	19	0	0	3
Delayed separation, 24-48 hr	20	0	0	0	0	0	0	0	0	0	13	0	3	0	8
Hemolysis, Hb >350 mg/dL	20[c]	11	0	2	0	0	1	6	0	0	6	0	5	2	0
Lipemia	20	2	0	1	1	8	0	5	0	1	2	4	1	0	8
Protein abnormalities															
Albumin, 1.4-4.0 g/dL	20	0	0	0	0	0	0	0	0	0	2	2	0	1	7
Protein, 7-18 g/dL	20	1	0	0	8	0	0	0	0	0	7	2	0	0	17
Protein, 3.1-6.2 g/dL	20	0	0	0	1	0	0	0	0	0	1	1	0	0	2
Kidney disease															
Pre-dialysis	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0
eGFR 15-30 mL/min/1.73m^2	19	0	0	1	1	0	0	0	0	0	1	0	0	0	1
Post kidney transplant	20	0	0	0	0	0	0	0	0	0	0	0	0	0	4
Protein, urine, 15 were 3-22 g/L	18	0	0	0	1	0	0	0	0	0	0	0	0	0	1

Manuscript in preparation

For IVD Manufacturers

- Implement calibration traceability to IDMS
  - SRM 967 is now available from NIST
  - Collaborate with a reference laboratory that offers IDMS measurements
    - JCTLM has approved 3 GC-IDMS methods and 1 LC-IDMS
    - Website: www.bipm.fr/en/committees/jc/jctlm/
- Address imprecision and non-specificity
- Communicate with PT/EQAS providers

Equations for estimating GFR

NKDEP recommends:

MDRD equation (1999, 2006 for IDMS calibration) to estimate GFR

GFR (mL/min/1.73 m^2) = 175 X (Serum Creatinine)$^{-1.154}$ X (Age)$^{-0.203}$ X (0.742 if female) X (1.210 if African American)

CKD-EPI (2009 - *Ann Intern Med* 2009;150:604)

- More accurate vs. measured GFR
- Consistent across 26 studies and subgroups (age, gender, race, diabetes, transplant status, BMI)

NIST SRM 967 for Creatinine Measurement

- Fresh-frozen human serum pools prepared according to CLSI C-37A
- Two levels:
  - **0.75 mg/dL (66 µmol/L)** – normal range - prepared from female donors without additives
  - **3.92 mg/dL (343 µmol/L)** – chronic kidney disease - prepared using supplementation with reagent grade creatinine
- Materials value assigned by NIST using LC-IDMS

Tools for creatinine measurement standardization

- US National Institute of Standards and Technology
  - ID-LC/MS Reference Measurement Procedure
- NIST Standard Reference Material (SRM 967a)
  - Fresh-frozen human serum pools prepared according to CLSI C-37A
  - Two levels:
  - **0.847 mg/dL (74.9 µmol/L)**
  - **3.88 mg/dL (343 µmol/L)**
- CAP LN24 Creatinine Accuracy/Linearity Survey
  - Pools value-assigned by ID-LC/MS RMP
- NIST SRM967 & CAP LN24 pools are commutable

NKDEP/NIST Commutability Study

Materials Evaluated (continued):

- NIST SRM 967 – 2 levels: 0.742 mg/dL (65.3 µmol/L) & 3.845 mg/dL (338.4 µmol/L)
- CAP LN24 Linearity Survey materials – 4 levels
  - LN24-2, "base pool", fresh frozen female serum pool without additives
  - LN24-7, "base pool" supplemented with reagent grade creatinine to obtain ~4 mg/dL (352 µmol/L)
  - LN24-3, blend of LN24-2 and LN24-7
  - LN24-1, LN24-2 diluted with phosphate buffered saline

NKDEP/NIST Commutability Study

Protocol:

- 20-24 patient serum samples, 2 levels of SRM 967, and 4 levels of CAP LN24 were analyzed by each field method in triplicate in a single analytical batch run
- Same set of patient samples and reference materials were analyzed by NIST using LC-IDMS (single measurement)
- Mean of the field method was plotted (y-axis) vs the reference method result (x-axis)

NKDEP/NIST Commutability Study

Protocol (continued):

- A least squares linear regression line and parameters including Sy.x were computed from the patient data
- Location of data points for the reference materials in relation to the 95% prediction interval (PI) around the regression line for the patient sample means were compared
- Commutability was defined as all points from a field method for a reference material must fall within the 95% PI defined by the patient serum data points

NKDEP

Summary of Commutability Study

- **SRM 967 has demonstrated commutability with the methods evaluated**
  - **Those methods found to be commutable will be listed on the NKDEP website**
- **CAP LN24 Linearity Survey materials appear to be commutable with the methods evaluated**