

# Standardization of Serum Creatinine and Estimated GFR in the Kidney Early Evaluation Program (KEEP)

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**Background:** Creatinine calibration by clinical laboratories is important because variability among assays adversely affects the accuracy of glomerular filtration rate (GFR) estimation. We describe the calibration of creatinine assays used in the National Kidney Foundation Kidney Early Evaluation Program (KEEP).

**Methods:** Creatinine values were requested for 200 samples at each of the 2 KEEP laboratories, Satellite Laboratory Services, LLC (2000 to 2005) and Clinical Laboratory Services (CLS; 2005 to present), for comparison with samples at the Cleveland Clinic Research Laboratory (CCRL). Linear regression and Deming regression were used to obtain slopes adjusted for measurement error and regression to the mean.

**Results:** After exclusion of outliers, mean creatinine level in 184 samples was 0.94 mg/dL at Satellite compared with 0.89 mg/dL at CCRL. Deming regression showed a slope of 1.003 (95% confidence interval (CI), 0.99 to 1.02;  $P < 0.001$ ) and intercept of  $-0.04$  (95% CI,  $-0.59$  to  $-0.02$ ;  $P = 0.003$ ) with  $R^2 = 0.9853$ . Final calibration consists of intercept alone because of a small slope. After exclusion of outliers, mean creatinine level in 199 samples was 1.06 mg/dL at CLS compared with 0.96 mg/dL at CCRL. Deming regression showed a slope of 1.08 (95% CI, 1.07 to 1.09;  $P < 0.001$ ) and intercept of  $-0.18$  (95% CI,  $-0.19$  to  $-0.17$ ;  $P < 0.001$ ) with  $R^2 = 0.9939$ . GFR estimates were minimally affected by the Satellite calibration. At a serum creatinine value of 1 mg/dL, the change in estimated GFR was 1 mL/min/1.73 m<sup>2</sup> after calibration. Conversely, higher range GFR estimates were affected by calibration of the CLS creatinine assay. At a serum creatinine value of 1 mg/dL, the GFR estimate was 6 mL/min/1.73 m<sup>2</sup> higher after calibration.

**Conclusion:** Calibration of KEEP creatinine measurements had a greater impact on the current laboratory than on the laboratory previously used. The calibration process has worked to decrease overestimation of eGFR at the high range and decrease misclassification bias.

*Am J Kidney Dis* 51(S2):S77-S82. © 2008 by the National Kidney Foundation, Inc.

**INDEX WORDS:** Calibration; creatinine; glomerular filtration rate (GFR) estimates.

Kidney function is assessed best by means of level of glomerular filtration rate (GFR). GFR is difficult to measure directly and therefore usually is estimated from serum levels of endogenous markers. Serum creatinine is the most common measure used to estimate level of kidney function; however, serum level is affected by factors other than GFR, including tubular secretion, generation, and extrarenal elimination. Variation in creatinine generation by age and race are well documented, largely caused by differences in muscle mass and diet. Estimating equations can overcome some of this variation by incorporating age, race, and sex into the creatinine-based assessment. Estimated GFR (eGFR) has become widely adopted in research studies and clinical practice. The most commonly used equations to estimate GFR are the Modification of Diet in Renal Disease (MDRD) Study and Cockcroft-Gault equations.<sup>1-3</sup>

It now is well recognized that use of creatinine assays different from the assay used in the clinical laboratory at which the equation was developed can substantially affect eGFR accuracy.

Variability among laboratories in serum creatinine assays is well known,<sup>4,5</sup> and calibration therefore is important for optimal results.

The Kidney Early Evaluation Program (KEEP) is a free community-based health-screening program sponsored by the National Kidney Foundation with the help of local National Kidney Foundation affiliates and volunteers. KEEP is designed to raise awareness about chronic kidney disease and prevent its complications by providing testing and information. This report describes KEEP methods of serum creatinine assay calibration to creatinine reference stan-

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0272-6386/08/5104-0110\$34.00/0

doi:10.1053/j.ajkd.2008.01.001

**Table 1. Methods Used for Measurement of Serum Creatinine in Kidney Early Evaluation Program Laboratories**

Laboratory	Instrument	Assay
Satellite Laboratory Services	Olympus 400-640	Kinetic alkaline picrate
Clinical Laboratory Services	Abbott Aeroset or Architect c8000	Aeroset c8000 creatinine assay, alkaline picrate

dards at the MDRD Study laboratory at the Cleveland Clinic Research Laboratory (CCRL) and aims to assess the impact of this calibration on the range of GFR estimates.

## METHODS

### Calibration

#### Laboratory Settings

KEEP was officially launched in August 2000. The program used Satellite Laboratory Services, LLC (SLS) from 2000 to 2005 and Clinical Laboratory Services (CLS) from November 1, 2005, through the present. Creatinine values were requested for 200 samples at each laboratory.

#### Serum Creatinine Assays at the CCRL

Calibration was established by comparing creatinine values measured at these laboratories with values measured at the CCRL. The CCRL used the modified kinetic rate Jaffé reaction (Beckman Synchron CX3; GMI, Inc., Ramsey, MN) method during the MDRD Study. The Beckman CX3 and Roche enzymatic method (Roche-Hitachi P-Module instrument with Roche Creatininase Plus assay; Hoffman-La Roche, Ltd., Basel, Switzerland) currently are used there. The Roche enzymatic assay at CCRL was shown to be equivalent to creatinine reference standard methods traceable to materials with values assigned by means of isotope dilution mass spectrometry. The Beckman Synchron CX3 assay also was calibrated to these creatinine reference standard methods, and the MDRD Study equation was re-expressed for use with standardized values. Details of the methods and results were described previously.<sup>6,7</sup> Therefore, calibration for KEEP specimens was performed on the Roche enzymatic assay, and calibration results are expressed as isotope dilution mass spectrometry-standardized values. The Roche assay showed coefficients of variation of 1.1% and 1.6% at creatinine values of 3.84 and 1.00 mg/dL (339 and 88.4  $\mu\text{mol/L}$ ) in 2005 (n = 409), respectively.

#### Calibration of Serum Creatinine Assays

SLS and CLS were surveyed for the instrument and assay used for creatinine measurement. A random sample of 200 specimens from each laboratory was requested for assay on the Roche enzymatic at CCRL. A sample size of 200 was

**Table 2. Comparison of Serum Creatinine Values at the CCRL and KEEP Laboratories**

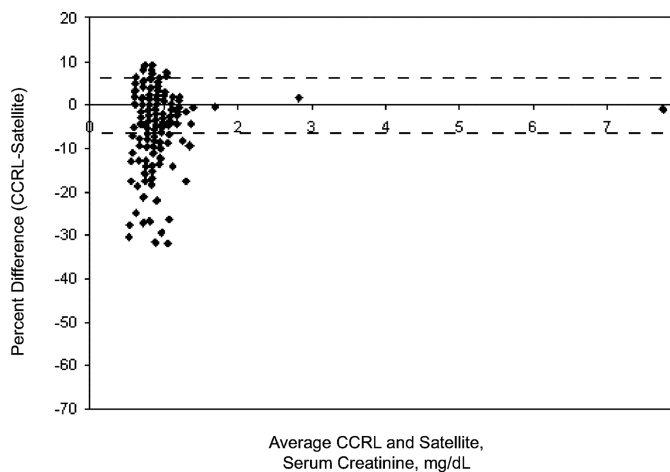
KEEP Laboratory	No. of Samples	Serum Creatinine (mg/dL)		Difference* CCRL-Lab		Percent Difference CCRL-Lab†	
		CCRL	KEEP Laboratory	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range
Satellite Laboratory Services							
Complete data	191	0.89 (0.46 to 7.72)	0.95 (0.6 to 7.8)	-0.05 $\pm$ 0.08	-0.33 to 0.08	-6.25 $\pm$ 11.85	-63.27 to 9.09
Excluding outliers	184	0.90 (0.46 to 7.72)	0.94 (0.6 to 7.8)	-0.04 $\pm$ 0.07	-0.29 to 0.08	-4.70 $\pm$ 8.74	-31.87 to 9.09
Clinical Laboratory Services							
Complete data	226	0.96 (0.52 to 8.18)	1.04 (0.6 to 7.8)	-0.12 $\pm$ 0.06	-0.26 to 0.38	-12.25 $\pm$ 6.17	-28.75 to 4.87
Excluding outliers	199	0.96 (0.58 to 8.18)	1.06 (0.6 to 7.8)	-0.10 $\pm$ 0.06	-0.20 to 0.38	-12.48 $\pm$ 6.21	-23.46 to 4.65

Note: Values expressed as mean (range), mean  $\pm$  SD, or range. To convert serum creatinine in mg/dL to  $\mu\text{mol/L}$ , multiply by 88.4.

Abbreviations: CCRL, Cleveland Clinic Research Laboratory; KEEP, Kidney Early Evaluation Program.

\*CCRL-Lab.

†((CCRL-Lab)/CCRL)  $\times$  100.



**Figure 1.** Bland-Altman plot for comparison of Satellite Laboratory Services versus Cleveland Clinic Research Laboratory (CCRL) after exclusion of outliers. Dashed lines, 1 SD. To convert serum creatinine in mg/dL to  $\mu\text{mol/L}$ , multiply by 88.4.

requested to allow for an SE for the difference between the original and new measurements of less than 0.02 mg/dL (1.8  $\mu\text{mol/L}$ ).

#### GFR Estimation

GFR was estimated by using the equation:  $\text{GFR} = 186 \times \text{original serum creatinine}^{-1.154} \times \text{age}^{-0.203} \times 1.212$  (African Americans)  $\times 0.742$  (women) for the original serum creatinine values and using the reexpressed MDRD Study equation for calibrated serum creatinine values<sup>7</sup>:  $\text{GFR} = 175 \times \text{standardized serum creatinine}^{-1.154} \times \text{age}^{-0.203} \times 1.212$  (African Americans)  $\times 0.742$  (women).

#### Statistical Analyses

Linear regression was used to assess the significance of the intercept and slope. Slopes and intercepts were adjusted to account for measurement error and regression to the mean by using Deming regression.<sup>8,9</sup> The presence of outliers was determined initially by visual inspection and, if present, defined as 3 SDs of the absolute percentage of difference. Calibrated creatinine measurements were calculated at values of 1.0 mg/dL (88  $\mu\text{mol/L}$ ) and 3.0 mg/dL (264  $\mu\text{mol/L}$ ).

Analyses were carried out using SAS software (version 9.1; SAS Institute, Cary, NC). Figures and Deming regressions were calculated in Excel Analyse-it (version X; Microsoft Corp, Redmond, WA).<sup>8,9</sup>

## RESULTS

#### Creatinine Calibration

Both SLS and CLS used the kinetic alkaline picrate methods (Table 1).

A total of 200 SLS samples from 7 KEEP programs in 7 cities in 3 states was sent to CCRL in October 2005. Of these, 191 were available for analysis. Table 2 and Fig 1 show the distribution of results from comparison of creatinine measurements at both sites. The mean difference was  $-0.05 \pm 0.08$  (SD) mg/dL ( $-4 \pm 7$   $\mu\text{mol/L}$ ).

Seven outliers were removed from the analysis, leaving 184 samples for calibration. Table 2 and Fig 1 show results of the comparison after exclusion of outliers.

Table 3 lists results of the CCRL regression on SLS values before and after exclusion of outliers for both linear and Deming regression. The final CCRL Deming regression showed a slope of 1.003 (95% confidence interval [CI], 0.99 to 1.02;  $P < 0.001$ ) and intercept of  $-0.04$  (95% CI,  $-0.59$  to  $-0.02$ ;  $P = 0.003$ ) with  $R^2 = 0.9853$ . Given the small slope, the final calibration factor consists of only an intercept adjustment.

A total of 226 CLS samples from 7 KEEP programs in 7 cities in 3 states was sent to CCRL in October 2005. Table 2 and Fig 2 show the distribution of results from the comparison of creatinine measurements at both sites. The mean difference was  $0.12 \pm 0.06$  (SD) mg/dL ( $11 \pm 5$   $\mu\text{mol/L}$ ). Twenty-seven outliers were removed from the analysis, leaving 199 samples for calibration. Table 2 and Fig 2 show results of the comparison of creatinine values after exclusion of outliers.

Table 3 lists results of the regression of the CCRL on CLS values before and after exclusion of outliers for both linear and Deming regression. Final Deming regression showed a slope of 1.08 (95% CI, 1.07 to 1.09;  $P < 0.001$ ) and intercept of  $-0.18$  (95% CI,  $-0.19$  to  $-0.17$ ;  $P < 0.001$ ) with  $R^2 = 0.9939$ .

#### GFR Estimation

The creatinine assay at the SLS was close to the CCRL assay; thus, the effect on eGFR using

Table 3. Calibration Factors

Laboratory	Intercept $\pm$ SE	Slope $\pm$ SE	R <sup>2</sup>
Satellite Laboratory Services			
Complete data			
Linear regression	-0.03 $\pm$ 0.0116	0.99 $\pm$ 0.0107	0.9784
Deming regression	-0.05 $\pm$ 0.0116	1.011 $\pm$ 0.0107	0.9784
Excluding outliers			
Linear regression	-0.02 $\pm$ 0.0097	0.98 $\pm$ 0.009	0.9853
Deming regression	-0.04 $\pm$ 0.0097	1*	0.9853
Clinical Laboratory Services			
Complete data			
Linear regression	-0.19 $\pm$ 0.0077	1.07 $\pm$ 0.007	0.9915
Deming regression	-0.17 $\pm$ 0.0077	1.08 $\pm$ 0.007	0.9915
Excluding outliers			
Linear regression	-0.18 $\pm$ 0.0071	1.07 $\pm$ 0.006	0.9939
Deming regression	-0.18 $\pm$ 0.0071	1.07 $\pm$ 0.006	0.9939

Note: To convert serum creatinine in mg/dL to  $\mu$ mol/L, multiply by 88.4.

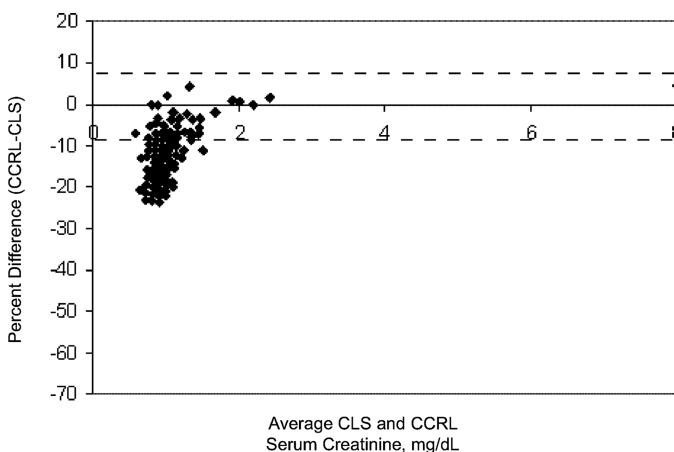
\*Slope was 1.003  $\pm$  0.009 (SE) and therefore considered to be small relative to the imprecision in the assay and dropped.

calibrated versus noncalibrated serum creatinine values was minimal (Fig 3A). For example, for serum creatinine values of 1 and 3 mg/dL (88 and 264  $\mu$ mol/L), the GFR estimate using the calibrated creatinine values was 1 mL/min/1.73 m<sup>2</sup> (0.02 mL/s/1.73 m<sup>2</sup>) less than the estimate using uncalibrated values. Conversely, the effect of calibration at CLS at the higher range of GFR was larger, decreasing to near 0 around 60 mL/min/1.73 m<sup>2</sup> (1.0 mL/s/1.73 m<sup>2</sup>; Fig 3B). For example, for serum creatinine values of 1 and 3 mg/dL (88 and 264  $\mu$ mol/L), GFR estimates using calibrated creatinine values were 6 mL/min/1.73 m<sup>2</sup> (0.1 mL/s/1.73 m<sup>2</sup>) higher and 2 mL/min/1.73 m<sup>2</sup> (0.02 mL/s/1.73 m<sup>2</sup>) lower than estimates using uncalibrated values, respectively.

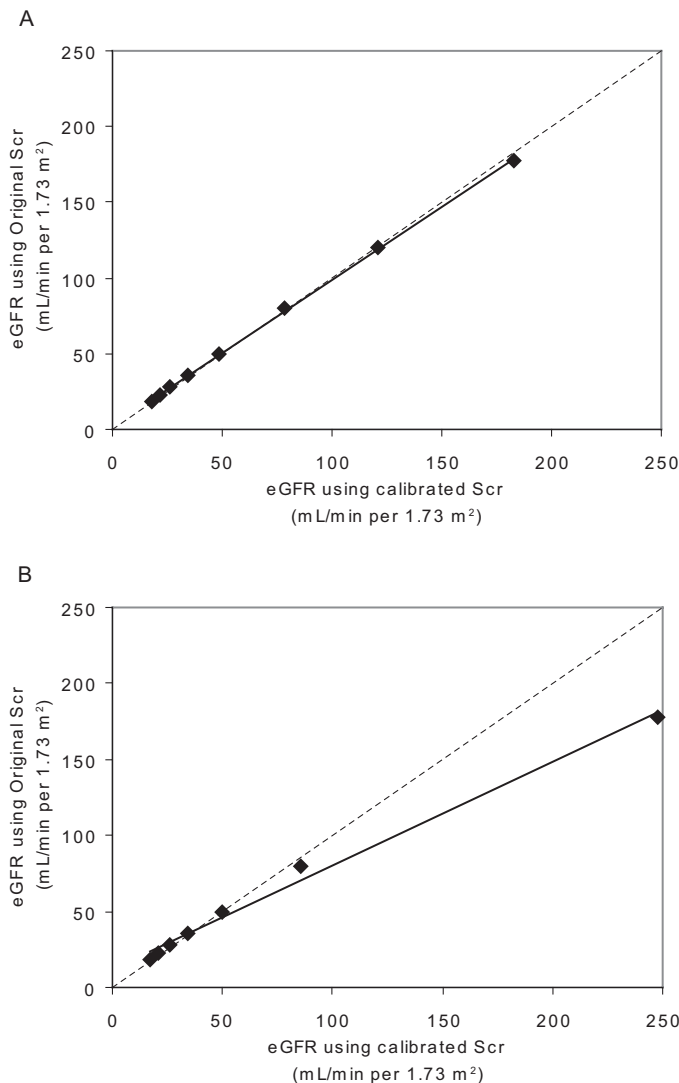
## DISCUSSION

We describe methods and results of calibration of the serum creatinine assay at the 2 laboratories used by KEEP. The key findings showed variation between laboratories in their comparison to creatinine reference standards and in the impact on eGFR.

For the SLS, creatinine instrument performance was similar to the Roche enzymatic assay at the CCRL, whereas results from the CLS laboratory showed substantial positive bias. This is consistent with the known heterogeneity of creatinine assays currently in use in the United States. A recent survey of the College of the American Pathologists (CAP) of 5,624 participating clinical laboratories in the United States



**Figure 2.** Bland-Altman plot for comparison of Clinical Laboratory Services (CLS) versus Cleveland Clinic Research Laboratory (CCRL) after exclusion of outliers. Dashed lines, 1 SD. To convert serum creatinine in mg/dL to  $\mu$ mol/L, multiply by 88.4.



**Figure 3.** Comparison of estimated glomerular filtration rate (eGFR) calculated from calibrated versus original serum creatinine (Scr) values. Points represent eGFR values for hypothetical 65-year-old white men with serum creatinine values of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 mg/dL. Dashed line, line of identity; black line, linear regression line through the points. (A) Satellite Laboratory Services, and (B) Clinical Laboratory Services. To convert GFR in mL/min/1.73 m<sup>2</sup> to mL/s/1.73 m<sup>2</sup>, multiply by 0.01667.

showed that mean bias varied from  $-0.06$  to  $+0.31$  mg/dL ( $-5$  to  $+27$   $\mu\text{mol/L}$ ) in 50 instrument-method peer groups. Bias variability was related to instrument manufacturer, rather than method type; 24 of 38 alkaline picric acid methods (63%) and 6 of 12 enzymatic methods (50%) showed significant biases.<sup>10</sup> Of interest, instruments and assays analogous to those used by SLS and CLS were found to have a pattern in magnitude of bias similar to the present study. The bias of the Olympus 400-640 (Olympus America, Inc., Center Valley, PA) using the kinetic alkaline picrate method in the CAP survey was 0.09 mg/dL (8  $\mu\text{mol/L}$ ), whereas in the present study, bias of a similar machine at the SLS was 0.04 mg/dL (4  $\mu\text{mol/L}$ ). Similarly, bias

of the Abbott Aeroset (Abbott Laboratories, Abbott Park, IL) using the kinetic picrate method in the CAP study was 0.14 mg/dL (12  $\mu\text{mol/L}$ ), and bias using a similar machine at the CLS laboratory was 0.1 mg/dL (9  $\mu\text{mol/L}$ ).

The impact of calibration on the accuracy of the GFR estimating equation is now well recognized.<sup>4,5,11</sup> Errors are larger at higher levels of GFR and although are smaller near 60 mL/min/1.73 m<sup>2</sup> (1.0 mL/s/1.73 m<sup>2</sup>), they can still cause substantial differences in estimated prevalence of CKD when applied to large populations.<sup>12-14</sup>

Because of the large variation among laboratories and instruments in calibration of creatinine instruments, anticipating the difference between one machine and the next is difficult. It is not

possible for all laboratories to calibrate to the CCRL or any other single laboratory. The National Kidney Disease Education Program of the National Institutes of Health established a program for standardization of all laboratories by 2008. Importantly, the program includes manufacturers; thus, all machines are expected to be calibrated to reference standards in the near future.

Limitations of this analysis include our use of samples from only 1 month to test each laboratory. This may be a particular problem for SLS, where KEEP measured creatinine for 5 years before calibration. However, laboratories generally are skilled at reducing drift over time, and the same instrument was in use during the 5 years. New creatinine instruments or assays used in the CLS laboratory should be tested going forward to maintain current standards.

In summary, calibration of the serum creatinine assay in KEEP has a larger effect in the laboratory currently in use than in the laboratory formerly used. Calibration improves the accuracy of estimating CKD prevalence in the overall program and detection of CKD in individual participants.

### ACKNOWLEDGEMENTS

The authors thank Frederick Van Lente, PhD, for measurement of creatinine at the CCRL and Edward Constantini, MA, Shane Nygaard, BA, and Nan Booth, MSW, MPH, of the Chronic Disease Research Group for figure preparation, manuscript preparation, and manuscript editing, respectively.

*Support:* The Kidney Early Evaluation Program is a program of the National Kidney Foundation Inc and is supported by Amgen, Abbott, Genzyme, Ortho Biotech Products LP, and Novartis, with additional support provided by Siemens Medical Solutions Diagnostics, Lifescan, Suplena, and OceanSpray Cranberries.

*Financial Disclosure:* Dr Stevens has received lecture fees from Quest Diagnostics. Dr Stoycheff has no conflicts of interest.

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