

Multicentre Evaluation of a Newly Developed Anti-Calcium Dobesilate-Enzymatic Creatinine Assay Based on Real-World Data in China

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Research

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Abstract

Background

Previously, we reported that calcium dobesilate (CaD), a vasoprotective agent mainly used for diabetic retinopathy, negatively interferes with enzymatic creatinine assays. A newly developed enzymatic creatinine assay, the “Cr-R assay”, is commercially available and claims to have no interference from CaD. This study aimed to verify the performance of the Cr-R assay on clinical samples and to investigate the degree of CaD interference in a multicentre, real-world study.

Methods

The precision and accuracy of the Cr-R assay was evaluated in 23 hospitals in different regions of China. Interference was then calculated both *in vitro* and in clinical samples. Samples with potential interference were screened based on the different creatinine results between Cr-R and the creatinine assays commonly used in each participating hospital (Cr-C). Interference was confirmed by determining serum CaD concentration by directly using ultra-performance liquid chromatography (UPLC) method and by assessing the “true” creatinine levels using the standard isotope dilution mass spectrometry (ID-MS/MS) method.

Results

The precision and bias and anti-interference ability of Cr-R assay on most platforms fulfilled the specification criteria. In the clinical setting, 67,469 samples were tested in 23 centres, and CaD concentration was measured in 818 samples, of which 257 contained CaD, accounting for 0.38% of the total and 31% of screened patients. In these 257 cases, the median CaD concentration was 11.356 (range, 1.350–121.519; IQR, 7.162–21.175) µg/mL. Creatinine levels measured using Cr-C assay were up to 35.1% (95%CI, 37.9–32.4 %; $P < 0.001$) lower than the “real” creatinine levels using the ID-MS/MS method. For Cr-R reagent, the average bias from the reference method was -6.2% (95%CI, -7.9–4.6%; $P < 0.001$), ranging from -17.9% to 7.1%.

Conclusions

Multicentre tests confirmed that the precision, bias, and anti-CaD ability of the Cr-R assay met clinical needs. The prevalence of CaD interference should be considered when performing clinical enzymatic creatinine measurements. Other manufacturers should improve the anti-interference performance of creatinine reagents.

Background

Creatinine is the basic indicator for monitoring the renal function of patients and the most critical index for calculating the estimated glomerular filtration rate in both prognosis and treatment monitoring. The traditional alkaline picrate method used to determine creatinine concentrations in the plasma or serum is

affected by interference from numerous endogenous and exogenous substances [1, 2]. Recently, the Trinder reaction-based enzymatic creatinine assay has been increasingly utilised because of its sensitivity and specificity and its compatibility with automated analysis [3]. According to the National Centre for Clinical Laboratories (NCCL) of China, more than 75% of clinical laboratories (2548/3116) in China used enzymatic assays to quantify creatinine in 2019 [4]. In recent years, manufacturers have made efforts to standardise their creatinine measurements to make the calibration traceable to reference methods, such as isotope dilution mass spectrometry/mass spectrometry (IDMS/MS), or to standard materials [5]. However, calibration traceability still cannot address issues such as interference.

As early as 1986, the vasoprotective agent calcium dobesilate (CaD, calcium 2,5-dihydroxybenzenesulfonate) was reported to interfere with enzymatic creatinine detection [2]. This issue was not resolved through additional research [6, 7]. CaD is a synthetic vasoprotectant that can remarkably reduce capillary permeability, blood viscosity, and high platelet activity, as well as improve abnormal hemorheology and microcirculation [8]. It is used to treat microangiopathies, particularly diabetic retinopathy (DR), worldwide [8-13]. Recent studies showed its protective effects on diabetic nephropathy and its ability to reduce albuminuria [14, 15]. Treatment strategies that include CaD may prevent or delay the progression of DR and nephropathy [16]. However, when CaD is used clinically to prevent and treat DR and/or DN, negative interference with enzymatic creatinine may lead to incorrect evaluation of renal function in patients with diabetes, which can mask the patient's condition and delay treatment, leading to poor clinical outcomes. Increased clinical application of CaD drugs has exacerbated the interference with creatinine measurements. In 2015, we reported clinically significant negative interference with CaD in eight Trinder reaction-based enzymatic creatinine assays [17]. According to our survey of 638 laboratory staff-members in different regions of China in 2019, 11% of respondents identified cases of creatinine interference caused by CaD. Thus, CaD interference in creatinine measurement is a common clinical problem that urgently requires resolution. Reducing the interference of the enzymatic creatinine reagent may be sufficient to overcome this issue.

In May 2019, an assay to measure enzymatic creatinine claiming no interference with CaD concentrations below 100 µg/mL became commercially available in China (referred to as the Cr-R assay). This is the first enzymatic creatinine reagent worldwide that claims to have no interference from CaD. The present study was conducted to verify the performance of this new Cr-R assay based on a multicentre investigation and real-world data. Because other commercially available creatinine assays (referred to as Cr-C assays) have significant negative interference with CaD, serum samples from patients being administered CaD contain the drug, resulting in a significantly lower creatinine value using traditional Cr-C assays. Clinical recommendations based on the present findings should guide decision making going forward, particularly in those patients with higher CaD levels.

Methods

Participating laboratories and experimental design

The present study was performed in 2019 and involved 23 clinical laboratories located in 23 hospitals from 15 cities in different regions of China. The biochemical analysers used in the 23 laboratories included the Abbott Architect (n = 1) (Abbott Diagnostics, Park City, IL, USA), Beckman AU (n = 15) (Beckman Coulter, Brea, CA, USA), Hitachi 7600 or 7180 (n = 3) (Tokyo, Japan), and Roche Cobas (n = 4) (Roche, Basel, Switzerland). The analytical performance of the Cr-R assays was evaluated on these 23 platforms. The Cr-R assays and calibrators were provided by Maccura Biotechnology (Chengdu, China). All reagents and calibrators used in this study were standardised across participating laboratories, with matching lot numbers. The detailed characteristics of the instruments and Cr-C assays used by the 23 participating laboratories are shown in Supplementary Table 1. Precision, method comparison and bias estimation, and anti-CaD ability of the Cr-R assay were evaluated based on the experimental workflow in Fig.1.

Preparation of serum samples

Pooled serum samples were prepared in a central laboratory (Department of Laboratory Medicine, Peking Union Medical College Hospital, PUMCH) and used to measure the precision of the different creatinine assays, as well as for method comparison, and *in vitro* interference evaluation. The creatinine concentrations of the low- and high-level serum pools used in the precision study were approximately 80 and 300 $\mu\text{mol/L}$, respectively. A panel of 40 serum pools were prepared for method comparison to cover a wide range of creatinine concentrations, from 40–1000 $\mu\text{mol/L}$. The creatinine concentrations of the three base serum pools used for the *in vitro* interference assessment were approximately 80, 133, and 442 $\mu\text{mol/L}$, respectively. The serum pools were fully mixed with non-icteric and non-haemolysed serum samples obtained from patients receiving physical examinations or who were hospitalised at PUMCH in June 2019; all patients had appropriate creatinine concentrations and no CaD. All pools were prepared within one week and frozen at $-80\text{ }^{\circ}\text{C}$ until shipped on dry ice to the reference laboratory (ID-MS/MS) and 23 participating clinical laboratories. The pools were stored in the participating laboratories at $-80\text{ }^{\circ}\text{C}$ until analysis.

Precision evaluation

The precision evaluation was performed according to Clinical and Laboratory Standards Institute (CLSI) EP15-A guidelines [18]. The high- and low-level serum pools were evaluated using the Cr-R assay four times daily for five consecutive days in each of the 23 participating clinical laboratories. An internal quality control was included to ensure test quality. The standard deviation (SD) and coefficient of variation (%CV) were calculated to determine the repeatability (within-run precision) and total precision (within-lab precision) for each assay. The inter-laboratory variation in the 23 participating laboratories was also calculated.

The within subject (CV_I) (4.4%) and between-subject (CV_g) (14.3%) variation of creatinine from the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Biological Variation Database [19] was used for the assessment of the performance characteristics of the creatinine assays.

The optimal, desirable, and minimal analytical precision for creatinine were 1.1, 2.2, and 3.3%, respectively, calculated using the following formula [20]:

Optimal Imprecision as CV (%): $CV_{\text{optimal}} = 0.25 CV_I$; desirable imprecision as CV (%): $CV_{\text{desirable}} = 0.5 CV_I$; minimal imprecision as CV (%): $CV_{\text{minimal}} = 0.75 CV_I$.

Method comparison and bias estimation

The creatinine assays were compared according to CLSI EP9-A3 guidelines [21]. Forty samples were measured in the 23 laboratories using the Cr-R assay on their automated analysers in 5 days, with 8 samples per day. Each sample was tested twice and within 3 h of thawing. The target values of serum pools were assigned by a reference measurement based on isotope dilution liquid chromatography-tandem mass spectrometry (LC-IDMS/MS) in two joint committees on traceability in laboratory medicine-listed reference laboratories: the NCCL, China, and the Reference Laboratory of Maccura Biotechnology (Chengdu, China). The average of these two laboratory measurements was used as the target value for each sample.

Correlation studies for each creatinine assay and the reference method were performed using Passing-Bablok regression, and bias at the medical decision levels and the 95% confidence interval (CI) were calculated. We set 88.4 $\mu\text{mol/L}$, 133 $\mu\text{mol/L}$, 265 $\mu\text{mol/L}$, and 442 $\mu\text{mol/L}$ as the levels for medical decisions involving creatinine. The analytical performance specification applied in bias evaluation was based on the EFLM BV data [19]. The optimal, desirable, and minimal bias specification for creatinine were defined as $\pm 1.9\%$, $\pm 3.7\%$, and $\pm 5.6\%$, respectively, calculated using the following formula:

Optimal bias%: $\text{Bias}_{\text{optimal}} = 0.25 (CV_I^2 + CV_G^2)^{1/2}$; desirable bias%: $\text{Bias}_{\text{desirable}} = 0.5 (CV_I^2 + CV_G^2)^{1/2}$; minimal bias%: $\text{Bias}_{\text{minimal}} = 0.75 (CV_I^2 + CV_G^2)^{1/2}$.

***In vitro* interference study**

Three drug-free serum pools (Cr = 80, 150, 442 $\mu\text{mol/L}$) were spiked with a CaD standard to prepare a dose-response series according to CLSI EP7-A2 guidelines [22]. The final CaD concentrations in each sample were 0, 8, 15, 30, 60, and 100 $\mu\text{g/mL}$. The CaD standard (100573, 94.8%) used in *in vitro* experiments was purchased from the National Institutes for Food and Drug Control of China (Beijing, China). In each participating hospital, the CaD-containing serum series was analysed using the Cr-C and Cr-R assay in triplicate within one analytical run to obtain an average value. The percent bias at each concentration of CaD was calculated relative to that of the drug-free specimen. Based on the biological variation, the acceptable limit of deviation for the *in vitro* experiments was defined as $\pm 5.6\%$.

Interference study based on real-world clinical data

Twenty-three participating laboratories used the Cr-C and Cr-R assays to simultaneously detect creatinine in the remaining serum of the clinical samples for 3–5 consecutive days. Each laboratory evaluated

approximately 3000 samples, which is clinically required to detect creatinine. If the creatinine measurement according to the Cr-C assay was 10% lower than that of the Cr-R measurement, the remaining serum was collected and stored in aliquots at -80 °C. CaD levels were accurately measured in these serum samples at PUMCH as described below. Fifty samples were randomly selected from those samples containing CaD to measure the creatinine levels using the above reference method, and deviations between the results of the two assays and the reference method were calculated.

Serum CaD concentration measurement by ultra-performance liquid chromatography (UPLC)

CaD ($\mu\text{g/mL}$) levels were measured using UPLC at the Department of Laboratory Medicine, PUMCH. Chromatographic separation was performed on an ACQUITY UPLC® BEH C18 column (100 μm \times 2.1 mm, 1.7 μm beads, Waters, Milford, MA, USA). The mobile phase was HPLC-grade water (A) (0.3% trimethylamine and pH regulated to 3.5 with glacial acetic acid) and acetonitrile (B). Isocratic elution was performed at a flow rate of 0.6 mL/min. The detection wavelength was 305 nm, and the retention time was approximately 4 min. The low and high limits of quantitation were 0.977 and 250 $\mu\text{g/mL}$, respectively. The analytical recovery was $100 \pm 5\%$. Within-laboratory CVs ranged from 2.46% to 3.31%.

Statistical analysis

MedCalc statistical software (ver. 18.11.6; MedCalc Software, Ostend Belgium) and GraphPad Prism software (ver. 5.01; La Jolla, CA, USA) were used for statistical analysis. Continuous data are presented as the means and standard deviations or medians (range, interquartile range [IQR], 25–75%), as appropriate. Paired Student's *t* tests were used for all comparisons, with $P < 0.05$ indicating a statistically significant difference.

Results

Precision evaluation

The mean creatinine concentrations of the low and high-level serum pools as determined by the 23 Cr-R assays were $82.5 \pm 3.4 \mu\text{mol/L}$ and $309.4 \pm 7.2 \mu\text{mol/L}$, respectively. The inter-laboratory variation of the low- and high-level pooled samples were 4.2% and 2.3%, respectively. Repeatability (within-run precision) and total precision (within-lab precision) for each assay are shown in Fig. 2. The within-laboratory CV% of the Cr-R assays reached the minimal specifications (3.3%) for all assays, except for in three laboratories (Beckman AU5400-2, Beckman AU5800-9, Roche Cobas 702-1) for the low-level creatinine serum pool. The high-level creatinine serum pool had less overall variation among laboratories, and the repeatability of the 22 Cr-R assays reached the minimum specification of 1.1% (Fig. 2B). The within-laboratory CV% was 0.4–1.9% (Fig. 2D), fulfilling the specification criteria (2.2%).

Method comparison and bias estimation

Passing-Bablok regression analysis was performed for each creatinine assay and the LC-IDMS/MS method. Bias at the medical decision levels of the individual creatinine assay for each of the 23

participating clinical laboratories is presented in Fig. 3. The regression equation of the Passing-Bablok regression and 95%CI of bias at the four medical decision levels are shown in Supplementary Table 2 and 3, respectively. The optimal specification for bias (1.9%) was reached in three Cr-R assays (Beckman AU5400-2, Roche Cobas 702-2, Beckman AU5800-2), whereas the desirable goal for bias (3.7%) was reached in three Cr-R assays at all medical decision levels. The other seven Cr-R assays fulfilled the minimal specification criterion (5.6%) at all medical decision levels. The lowest creatinine concentration showed the largest bias for most Cr-R assays. At the medical decision level of 88.4 $\mu\text{mol/L}$, 34.8% (8/23) of Cr-R assays failed to reach the minimal bias specification of 5.6% (Fig. 3A). At the medical decision level of 265 and 442 $\mu\text{mol/L}$, all Cr-R assays showed the desirable bias specification of 5.6% except for the Beckman AU5400-4 (Fig. 3C, D).

***In vitro* interference study**

The results of the *in vitro* interference test are shown in Fig. 4. Deviations in the Cr-R assays in determining the median and high-Cr interference pools were -2.5% to 4.95% and -5.2% to -0.12%, respectively, both meeting the minimal bias specification of 5.6% (Fig. 4B, C). For low-Cr interference pools, deviations in the Cr-R assays ranged from 1.82% to 15.95% (Fig. 4A). Exogenous addition of CaD clearly exhibited concentration-dependent negative interference in creatinine measurement in the Cr-C assays in all of the 23 participating laboratories. When the CaD concentration was 100 $\mu\text{g/mL}$, the percentage bias relative to the drug-free specimen of the low, median, and high creatinine concentration interference serum samples according to Cr-C ranged from -77.78% to -38.55%, -71.47% to -33.33%, and -58.72% to -22.47%, respectively (Fig. 4A–C).

Interference study based on real-world data

A total of 67 469 samples were evaluated using the Cr-R and Cr-C assays simultaneously to measure creatinine concentrations in 23 participating clinical laboratories. Following this, 818 samples were screened out because the results of the Cr-C assay were 10% lower than the Cr-R measurement. CaD was detected in the serum of 257 patients out of the screened samples, accounting for 0.39% of all patients and 31% of the screened samples. In these 257 cases, the median CaD concentration was 11.356 (range, 1.350–121.519; IQR, 7.162–21.175) $\mu\text{g/mL}$. The median level of creatinine according to the Cr-C and Cr-R assays were 94.8 $\mu\text{mol/L}$ (range, 20.0–1316.2; IQR, 57.2–159.0) and 119.4 $\mu\text{mol/L}$ (range, 41.0–1615.0; IQR, 72.0–202.9), respectively.

Fifty samples were randomly selected from the CaD-containing samples to accurately measure creatinine levels by LC-IDMS/MS. A higher serum creatinine level was associated with a higher CaD concentration (Fig. 5A). Thus, creatinine levels according to the Cr-C assay were up to 35.1% (95% CI, 37.9 to 32.4%; $P < 0.001$) lower than the actual creatinine levels. For Cr-R reagent, the average bias from the reference method was -6.2% (95% CI, -7.9 to -4.6%; $P < 0.001$), ranging from -17.9 to 7.1% (Fig. 5B).

Discussion

To overcome the problem of CaD interference with enzymatic creatinine measurements, and thus reduce clinical risk, an enzymatic creatinine test, the Cr-R assay, claiming to have no interference from CaD below 100 µg/mL was introduced in China. In the present study, we investigated the basic analytical performance of the Cr-R assay on different platforms and in different hospitals. According to the 2014 EFLM European Biological Variation Study, the performance specifications of a clinical creatinine test depend on the within-subject (3.4%) and between-subject (14.3%) variation of creatinine [19], which were obtained from the latest data from the EFLM database. Pooled serum samples were used to determine the within-lab %CV variation of the Cr-R assay, with 3 out of the 23 participating laboratories not meeting the minimum standards on pooled samples. The status of each biochemical analyser, including the age of the sample loading stage, the use of the light source, and the cleanliness of the cuvettes may affect the precision of the results. The within-laboratory CV% of the laboratory using a Beckman AU5400-2 was as high as 4.5%. Further investigation revealed that the instrument had been in use for more than 6 years. In the present study, only 13 Cr-R assays fulfilled the minimal specification criterion (5.6%) at all medical decision levels. The accuracy of creatinine measurement at low levels therefore still remains a major challenge. From the summary report of the Creatinine Accuracy Calibration Verification/Linearity LN24-A 2019, organised by the College of American Pathologists, the allowable error at low creatinine levels is $\pm 14.5\%$, and at high creatinine levels it is $\pm 7.5\%$. When using the minimal specification criterion (5.6%) proposed by this study, the results from approximately half of the laboratories assessed in the LN24-A 2019 may not meet minimum standards for low creatinine levels. Although all 23 laboratories used the same lot of Cr-R reagents and calibrators, the bias obtained by different research centres varied widely, even when using the same model of biochemical analyser. This may have occurred for the following reasons: First, creatinine uses a single-point calibration, and any deviations, such as improper reconstitution of the calibrator or differences in temperature and time exposure when the calibrator is opened, may therefore cause systemic changes in the outcomes of the assay. Second, the large bias in some centres was caused by large day-to-day variation, which may be related to instrument maintenance or reagent stability. We instructed participating laboratories to operate according to their internal QA/QC guidelines, without requiring multiple calibrations for Cr-R assays. The large levels of variation obtained using standard reagents should therefore be alarming to laboratories indicating the need to give more attention to daily QC and calibration.

We also spiked pooled serum samples with increasing concentrations of CaD to evaluate alongside these pooled samples to determine the critical concentration at which CaD levels affect the results of the Cr-R and Cr-C assays. According to these results, CaD levels $> 8 \mu\text{g/mL}$ result in the under-representation of serum creatinine levels according to the Cr-C assay (Fig.4). For the Cr-R assay, increased CaD concentrations tended to show slightly higher values only in low-Cr interference pools, which was obvious at 60 µg/mL or higher CaD (Fig.4A). However, based on pharmacokinetic information (23) and actual measured results, when the patient's creatinine level is close to the low-Cr interference pools, the CaD concentration is not expected to reach 60 µg/mL (Fig. 5A). As the usual recommended dosage of CaD is 500 mg 3 times per day, the blood serum concentration is estimated to be 15 µg/mL at the steady state [23]. However, CaD is a small molecule that is primarily excreted through the kidneys. Patients with renal

failure have exceedingly low glomerular filtration rates and are thus prone to drug accumulation (Fig. 4A), which may result in higher serum CaD levels and thus, more severe interference with the results of the Cr-C assay (Fig. 4B). At the highest CaD concentration (121.519 µg/mL) in the screened samples, the creatinine levels according to the Cr-R assay did not show strong deviation (-5.2%) from the levels as determined by LC-IDMS/MS. In summary, the new Cr-R assay met clinical needs according to its ability to measure serum creatinine levels without interference from CaD.

When applying the Cr-R and Cr-C assays to a large clinical cohort comprised of 67 469 samples, we identified 818 patients with large differences in creatinine levels according to the Cr-R and Cr-C assays, while only one-third of these patients had detectable CaD in their serum. For the remaining samples, the observed differences may be partially attributable to systemic differences between different reagents or other unknown interference factors in the serum samples. A number of substances may interfere with creatinine quantification using enzymatic assays [24-29]. For some patients with large deviating Cr-R and Cr-C assay results who did not have detectable serum CaD, we evaluated the patient's medical records and compared the creatinine levels with those determined by the reference method and identified ten cases where this discrepancy may have been caused by the presence of etamsylate. Etamsylate is known to interfere with enzymatic creatinine assays [28-29]. The Cr-R assay also solved the problem of interference by etamsylate, and generally gave more accurate serum creatinine results in these cases. The manufacturer of the Cr-R assay claims that serum etamsylate levels below 250 µg/mL will not interfere with the assay results, and that the Cr-R assay may also work in the presence of other analogues, which interfere with enzymatic creatinine assays.

There were some limitations to the present study. First, in the precision assessment, we used EP15A but not the latest EP15A3 due to the volume limitation of the serum pools. Second, the serum pools used in the precision evaluation were not checked for homogeneity and freeze-thaw effects. However, previously published data showed that a freeze-thaw cycle does not alter the stability of creatinine in pooled serum [30].

Conclusions

Both *in vitro* addition tests and real-world data revealed that the new Cr-R assay can overcome clinical CaD interference with creatinine detection. Multicentre tests confirmed that the precision and accuracy meet the needs of clinical testing. Additionally, it is important to further raise awareness of such interference so that clinical physicians can make judgements on the levels of creatinine in patients under CaD therapy. Other manufacturers should improve the anti-interference performance of creatinine reagents.

Abbreviations

NCCL, National Centre for Clinical Laboratories; IDMS/MS, isotope dilution mass spectrometry/ mass spectrometry; CaD, calcium dobesilate; DR, diabetic retinopathy; PUMCH, Peking Union Medical College

Hospital; CLSI, Clinical and Laboratory Standards Institute; SD, standard deviation; CV, coefficient of variability; CV_i, within subject variation; CV_g, between-subject variation; LC-IDMS/MS, isotope dilution liquid chromatography-tandem mass spectrometry/ mass spectrometry; EFLM, European Federation of Clinical Chemistry and Laboratory Medicine; CI, confidence interval; BV, biological variation; UPLC, ultra-performance liquid chromatography

Declarations

Ethics approval and consent to participate

The study was approved by the local Ethical Committee of the PUMCH, Beijing (S-T750). Since the pools were prepared using remains of patient serum samples that were anonymised, it was not necessary to obtain written informed consent.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

None declared.

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Authors' contributions

Xiuzhi Guo, Li'an Hou, Zhongxin Lu, Guiru Zhi, Xiaoyan Li, Shiqing Cheng, Dexin Li, Yan Zhang, Xuejing Wang, Qi Li, Yufan Zhang, Aijun Niu, Dandan Wang, Peng Ren, Anchun Xu, Jianping Zhang, Yi Zhang, Guangming Qin, Honglan Yu, Wentao Liu, Xun Min, Suhua Hu, Shenghe Hu, Weiqing Song analysed and interpreted the patient data, and Xiuzhi Guo and Ling Qiu were major contributors to writing the manuscript. All authors read and approved the final manuscript.

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Figures

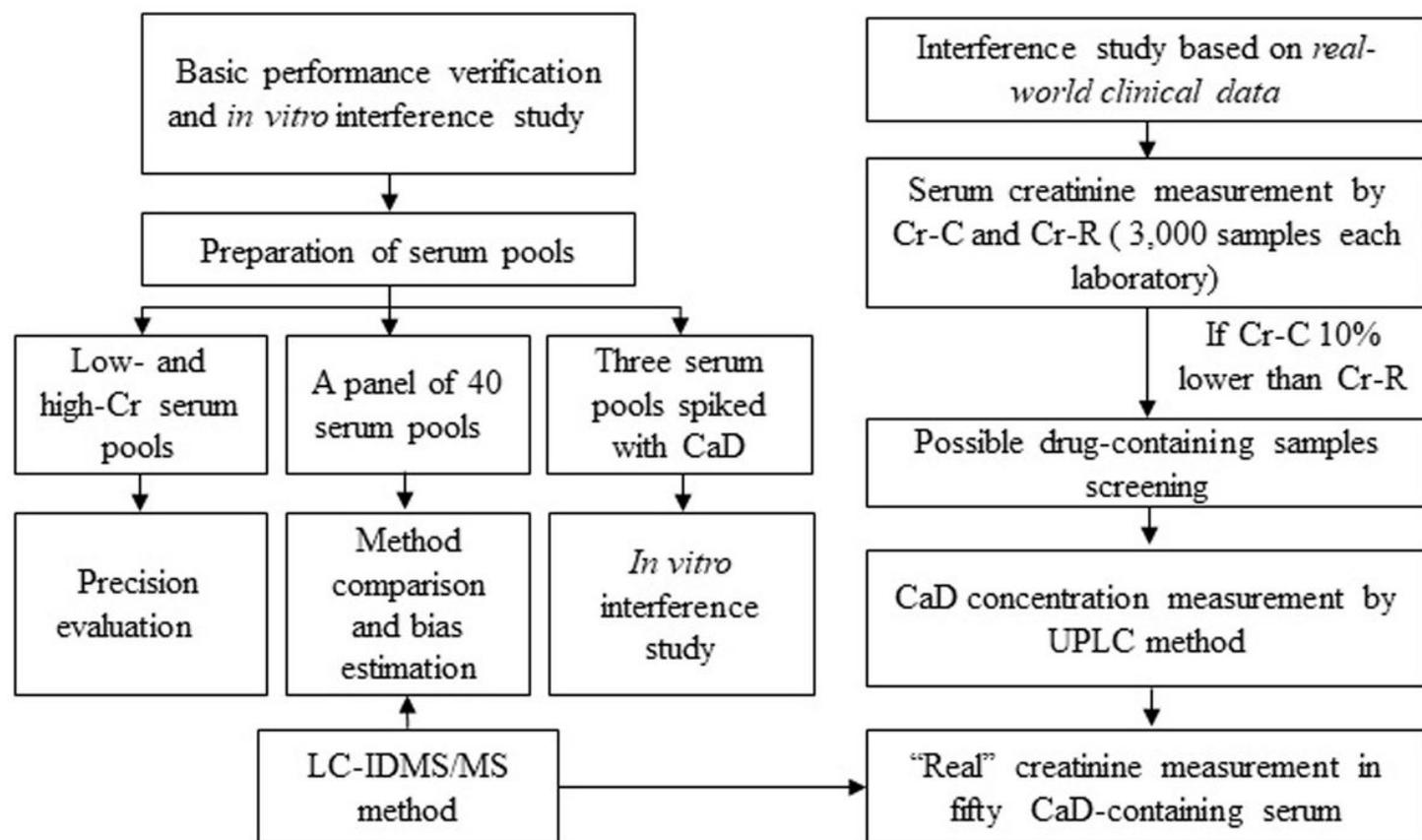


Figure 1

The experimental workflow of the study. LC-IDMS/MS, isotope dilution liquid chromatography-tandem mass spectrometry; UPLC, ultra-performance liquid chromatography.

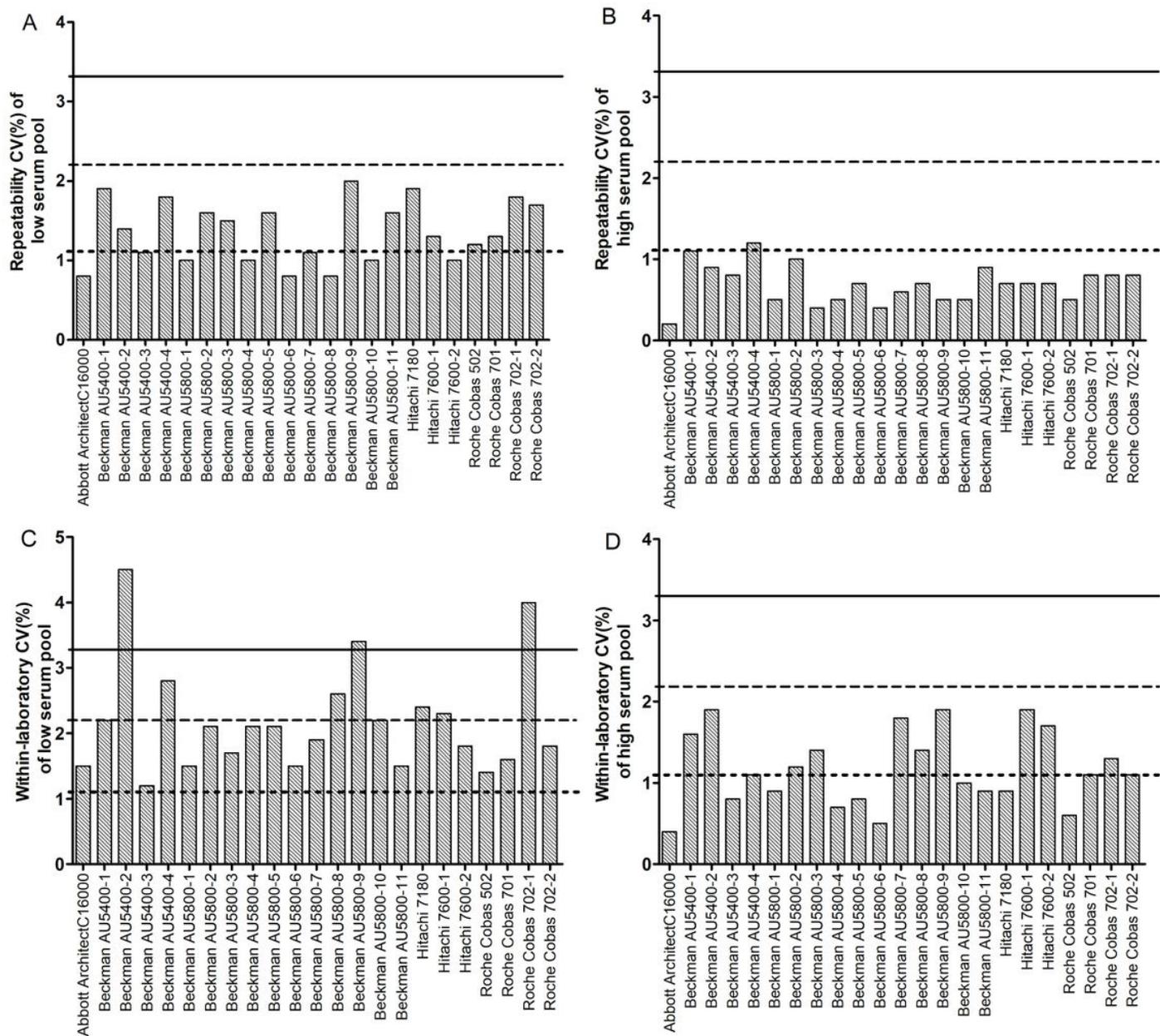


Figure 2

Repeatability according to CV% and within-laboratory CV% of Cr-R assays for pooled serum samples in 23 participating clinical laboratories. (A) Repeatability CV% of Cr-R assays in a pooled serum sample with low-Cr concentration (0.93 ± 0.04 mg/dL) (B) Repeatability CV% of Cr-R assays in a pooled serum sample with high-Cr concentration (3.50 ± 0.08 mg/dL); (C) Within-laboratory CV% for Cr-R assays in a low-Cr concentration serum pool; (D) Within-laboratory CV% for Cr-R assays in a high-Cr concentration serum pool. To convert creatinine concentrations from mg/dL to $\mu\text{mol/L}$, multiply by 88.4. The EFLM goals for clinical creatinine assays are indicated. Solid horizontal line: minimal (3.3%), long-dashed horizontal line: desirable (2.2%), short-dashed horizontal lines: optimal (1.1%).

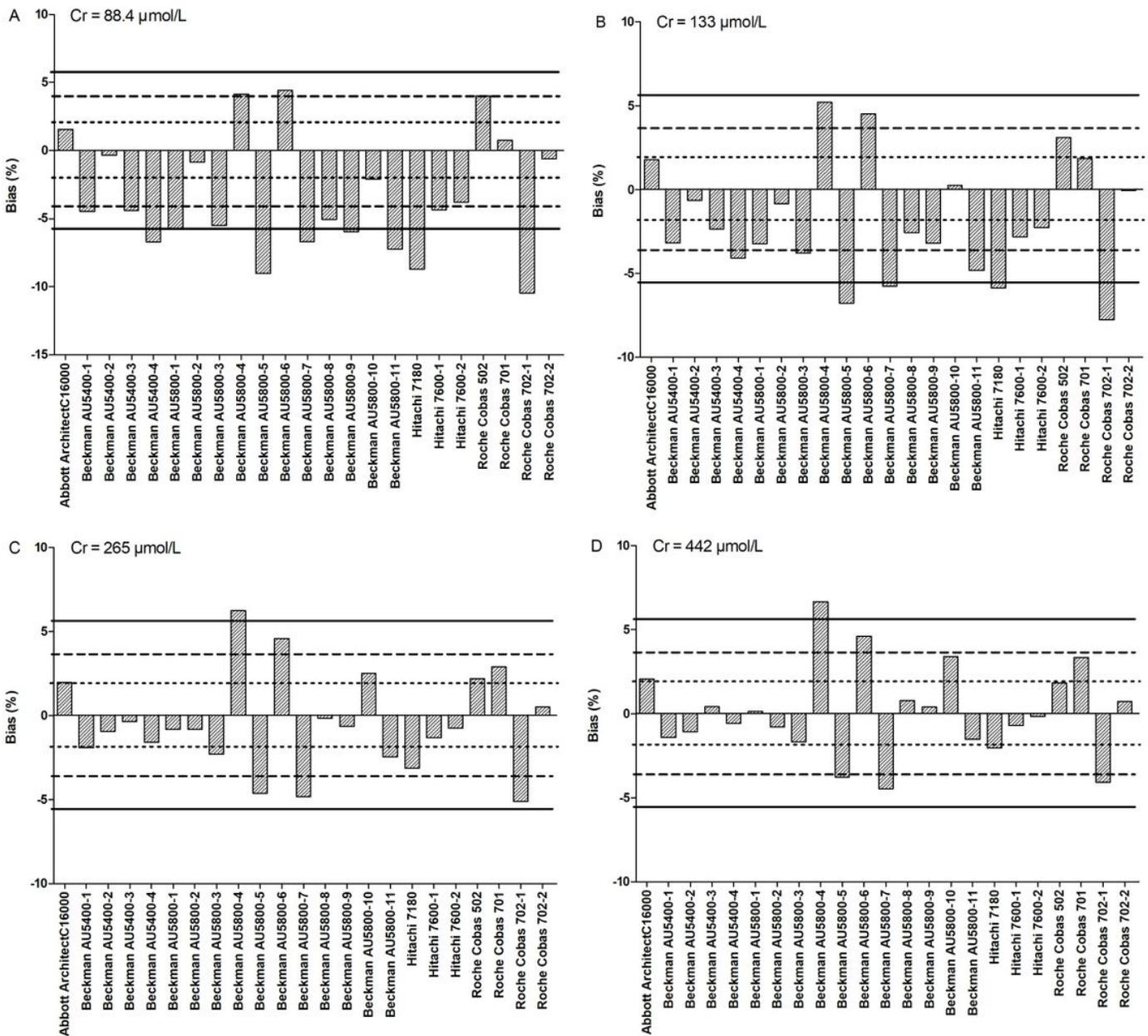


Figure 3

Bias at the medical decision levels for the levels of creatinine as determined by comparing the results of individual Cr assays and those of LC-IDMS/MS in each of the 23 participating clinical laboratories. (A) Medical decision level: 1 mg/dL; (B) Medical decision level: 1.5 mg/dL; (C) Medical decision level: 3 mg/dL; (D) Medical decision level: 5 mg/dL. To convert creatinine concentrations from mg/dL to $\mu\text{mol/L}$, multiply by 88.4. EFLM goals are indicated. Solid horizontal line: minimal ($\pm 5.6\%$), long-dashed horizontal line: desirable ($\pm 3.7\%$), short-dashed horizontal lines: optimal ($\pm 1.9\%$).

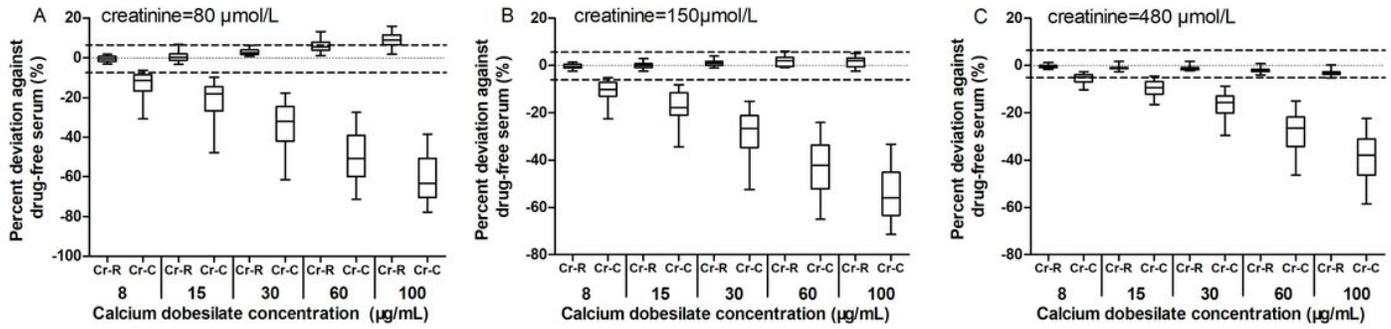


Figure 4

Box-and-whisker plot showing the percent deviation of creatinine concentrations according to Cr-R and Cr-C assays against CaD-free serum. (A) 23 Cr-R and Cr-C assays measuring creatinine levels in low-Cr concentration interference pools; (B) 23 Cr-R and Cr-C assays measuring creatinine levels in median-Cr concentration interference pools; (C) 23 Cr-R and Cr-C assays measuring creatinine levels in high-Cr concentration interference pools. To convert creatinine concentrations from mg/dL to $\mu\text{mol/L}$, multiply by 88.4. The boxes show the median, 25th, and 75th percentile values of bias for creatinine concentrations of 23 assays against drug-free serum. Whiskers extend from the minimum to the maximum value. Long-dashed horizontal line: desirable ($\pm 5.6\%$).

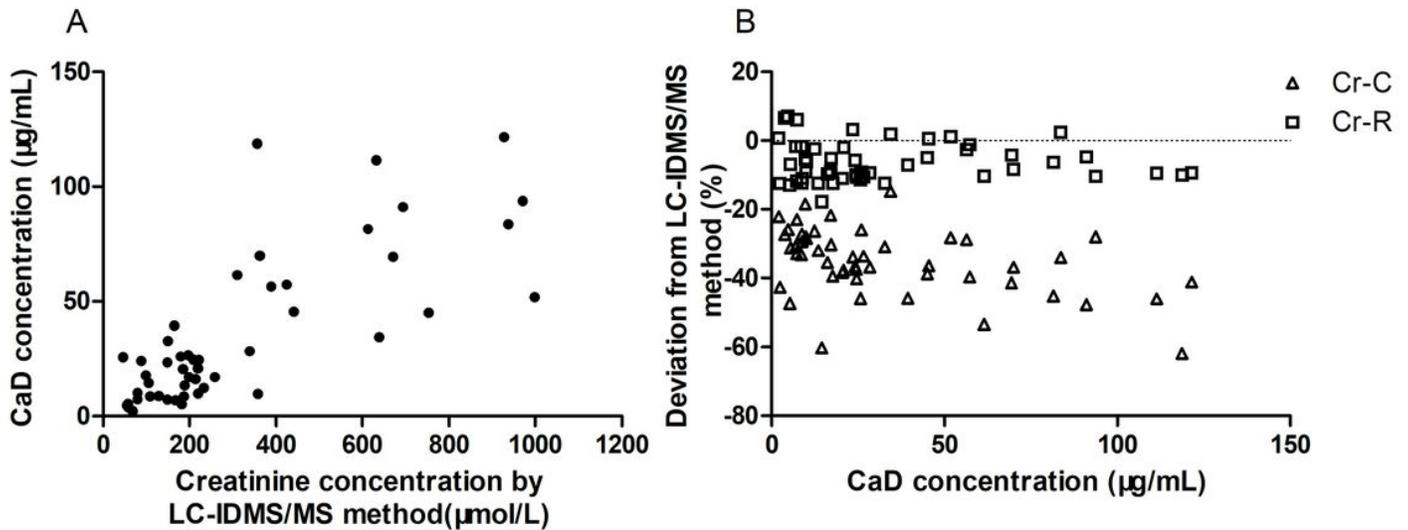


Figure 5

Scatter plots showing the CaD concentrations and creatinine measured by LC-IDMS/MS, Cr-C, and Cr-R assays. (A) CaD concentrations vs creatinine concentrations measured by LC-IDMS/MS. (B) Deviation of Cr-C and Cr-R assays from LC-IDMS/MS measurements of creatinine levels vs CaD concentrations.

Supplementary Files

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