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Multicenter Evaluation of Cystatin C Measurement after Assay Standardization

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BACKGROUND: Since 2010, a certified reference material ERM-DA471/IFCC has been available for cystatin C (CysC). This study aimed to assess the sources of uncertainty in results for clinical samples measured using standardized assays.

METHODS: This evaluation was performed in 2015 and involved 7 clinical laboratories located in France and Belgium. CysC was measured in a panel of 4 serum pools using 8 automated assays and a candidate isotope dilution mass spectrometry reference measurement procedure. Sources of uncertainty (imprecision and bias) were evaluated to calculate the relative expanded combined uncertainty for each CysC assay. Uncertainty was judged against the performance specifications derived from the biological variation model.

RESULTS: Only Siemens reagents on the Siemens systems and, to a lesser extent, DiaSys reagents on the Cobas system, provided results that met the minimum performance criterion calculated according to the intraindividual and interindividual biological variations. Although the imprecision was acceptable for almost all assays, an increase in the bias with concentration was observed for Gentian reagents, and unacceptably high biases were observed for Abbott and Roche reagents on their own systems.

CONCLUSIONS: This comprehensive picture of the market situation since the release of ERM-DA471/IFCC shows that bias remains the major component of the combined uncertainty because of possible problems associated with the implementation of traceability. Although some manufacturers have clearly improved their calibration protocols relative to ERM-DA471, most of them failed to meet the criteria for acceptable CysC measurements.

Cystatin C (CysC)[11] is an additional biomarker for the estimation of renal function and also for prediction of cardiovascular risk *(1, 2)*. Furthermore, recent recommendations from the Kidney Disease Foundation highlight the need to determine the estimated glomerular filtration rate (eGFR) based on creatinine and CysC ($eGFR_{Crea,CysC}$) used to confirm stage 3A (45–60 $mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}$) kidney disease in patients without any signs of kidney damage *(3, 4)*. The calibration and standardization of assays are key factors for developing eGFR equations based on CysC alone or in combination with creatinine *(5)*. Currently, a certified reference material, ERM-DA471/IFCC, is available for CysC *(6)*. With the availability of a reference material, improvement in the equivalence of the results from various assays would be expected.

Although some authors have shown good concordance between some specific assays *(7)*, a recent College of American Pathologists (CAP) survey showed that substantial variability between assays persists *(8)*. The 2014 CAP survey *(8)* was based on improved CAP assay coding, which allowed for identification of reagent/calibrator

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[11] Nonstandard abbreviations: CysC, cystatin C; SFBC, Société Française de Biologie Clinique; eGFR, estimated glomerular filtration rate; $eGFR_{Crea,CysC}$, estimated glomerular filtration rate based on creatinine and cystatin C; CAP, College of American Pathologists; RMP, reference measurement procedure; IDMS, isotope dilution mass spectrometry; CE, Conformité Européene; u_c, combined standard uncertainty; U_c, combined expanded uncertainty; u_{Bias}, standard uncertainty associated with the bias; u_R, standard uncertainty associated with random effect; S_r, repeatability SD; S_{Rw}, within-lab precision; S_R, reproducibility; JCTLM, Joint Committee for Traceability in Laboratory Medicine; EFLM, European Federation of Clinical Chemistry and Laboratory Medicine; IVD, in vitro diagnostics; $eGFR_{Crea}$, eGFR based on creatinine.

groups and used survey materials expected to be commutable, although commutability was not validated. A variety of instrument platforms and reagents were represented, and geographical variations in the calibration among specific groups could partially explain the variability in their results. To overcome these limitations, we used fully documented assays, and commutable materials with target values determined using a candidate reference measurement procedure (RMP) *(9, 10)* to evaluate the analytical performance of most of the available assays for CysC. Standard and expanded uncertainties were calculated by considering the uncertainties associated with bias and random effects (imprecision).

Materials and Methods

EXPERIMENTAL DESIGN

This evaluation was performed in 2015 and involved 7 clinical laboratories located in France and Belgium. CysC was measured in a panel of 4 off-the-clot fresh-frozen serum pools using 8 automated assays and an isotope dilution mass spectrometry (IDMS) candidate RMP. The commutability of the frozen pools was verified by measuring CysC in 30 fresh serum samples whose concentrations bracketed those of the 4 frozen pools. Each automated assay, which was composed of a combination of a reagent, calibrator, and analyzer, was assessed independently by 2 different laboratories.

The 30 serum samples were transported to each laboratory at 4 °C and analyzed in triplicate on 1 day within 4 days after selection. Simultaneously, frozen serum pools were sent on dry ice to the laboratories where they were stored at −20 °C until analysis. The frozen pools were first assayed on the same day as the 30 fresh serum samples to verify commutability and then on 3 separate runs per day over 3 consecutive days.

SERUM SAMPLES

The selection of clinical specimens for pool fabrication and for evaluation of commutability were based on increasing creatinine values measured during a routine examination. The pools were prepared from residual sera of at least 15 patients not receiving medication and that were not lipemic, icteric, or hemolyzed. The recommendations of the CLSI C37-A guideline *(11)* were observed for making the pools. Briefly, blood was collected into plain tubes, and allowed to clot for 1 h at room temperature. Tubes were centrifuged at 2500*g* for 10 min, with serum recovered and creatinine measured as soon as possible. Tubes were stored at 4 °C before selection of donors and before pooling. Pools were prepared from donor units with creatinine values ranging from 30–40 μmol/L (0.3–0.4 mg/dL) (pool 1), 70–80 μmol/L (0.8–0.9 mg/dL) (pool 2), 90–100 μmol/L (1.0–1.1 mg/dL) (pool 3), and 150–160 μmol/L (1.6–1.8 mg/dL) (pool 4). The pools were mixed gently for 12 h at 4 °C to ensure homogeneity, and 0.5 mL aliquots were transferred into the final containers. The aliquots were frozen at −80 °C. The collection, processing, dispensing, freezing, and storage processes of the pools were completed within 20 h. The same procedures were used to prepare 0.5 mL aliquots of the individual serum samples used for the commutability validation that were stored at 4 °C before distribution. Donor sera samples for commutability validation were selected to be within the bracketed creatinine concentrations of the 4 frozen pools. Because the study involved anonymized leftover samples, French law did not require approval by an Ethics Committee.

CysC ASSAYS AND REAGENT–CALIBRATOR–ANALYZER COMBINATIONS

Eight automated measuring systems (including the platform, calibrator, reagents, and control materials) were evaluated according to the manufacturer's instructions, and the calibration of each system was assessed in each run based on the acceptable ranges for the control materials specified by each manufacturer. Measuring systems from the same manufacturer were all Conformité Européenne (CE) marked. When a platform (Architect and Cobas c502) was used as an open system to implement alternative CE reagents/calibrators/controls (Gentian or DiaSys), the defined application for the CysC immunoassay was installed in accordance with the specific instrument's settings and the performance characteristics provided by the reagent's manufacturer. For open systems, the analyzer manufacturer's instructions were used for performing calibration and maintenance of the analyzer. The main characteristics of the 8 combinations of reagents, analyzers, and calibrators are summarized in Table 1.

CANDIDATE RMP

The target values of the 4 frozen pools were assigned in the Department of Physical and Analytical Chemistry (University of Oviedo, Spain) using an IDMS-based candidate RMP. The trueness of the candidate RMP was confirmed by recovering the assigned value for measurement of DA471/IFCC. The mean bias of the IDMS method was +1.0% relative to the certified value (n = 6). The uncertainty associated with the values assigned to the pools using the IDMS measurement procedure was assessed according to previously described methods *(9)*. Uncertainty sources were investigated by estimating the contribution of the uncertainty of each relevant parameter to the overall total combined uncertainty, as described by Kragten et al. *(12)*. The relative expanded uncertainties (k = 2) of the IDMS-based target concentrations were 3.8% for pool 1, 3.6% for pool 2, 1.9% for pool 3, and 2.9% for pool 4.

Table 1. Main characteristics of the 8 automated CysC reagent–analyzer combinations.

Reagents/analyzer	Participating laboratories	Principle underlying the commercial assay	CE marking	Antibody to human CysC (source)	Calibrator references, names, and concentrations	Declared expanded uncertainty (k = 2)	Stated traceability
Abbott (Abbott Laboratories) Multigent Cystatin C/Architect cSystem	Lyon Lab 1/Tenon	PETIA[a]	Yes (Abbott)	Polyclonal antibodies (goat) absorbed on latex particles	Cystatin C calibrator Ref 1P93 – 10 Lot 50148Y600: 9.97 mg/L	2.9%	ERM-DA471/IFCC
DiaSys (DiaSys Diagnostic Systems) Cystatin C FS/ Abbott Architect cSystem	Lyon Lab 1/enon	PETIA	Reagent/calibrator are CE marked (DiaSys) Application proposal is for guidelines only (should be approved by the biologist)	Polyclonal antibodies (goat) bound to carboxylated polystyrene particles	TruCal Cystatin C Ref 1 7150 99 10 059 Lot 20054: 0.50 mg/L Lot 20055: 1.50 mg/L Lot 20056: 3.00 mg/L Lot 20057: 5.50 mg/L Lot 20058: 8.00 mg/L	 4.6% 3.1% 3.3% 3.0% 3.3%	ERM-DA471/IFCC
DiaSys Cystatin C FS/Roche (Roche Diagnostics) Cobas c502	Avignon/Montpellier	PETIA	Reagent/calibrator are CE marked (Diasys) Application proposal is for guidelines only (should be approved by the biologist)	Polyclonal antibodies (goat) bound to carboxylated polystyrene particles	TruCal Cystatin C Ref 1 7150 99 10 059 Lot 20054: 0.50 mg/L Lot 20055: 1.50 mg/L Lot 20056: 3.00 mg/L Lot 20057: 5.50 mg/L Lot 20058: 8.00 mg/L	 4.6% 3.1% 3.3% 3.0% 3.3%	ERM-DA471/IFCC
Gentian Cystatin C/Abbott Architect cSystem	Lyon Lab 1/Tenon	PETIA	Yes The entire measurement system is CE marked (Gentian)	Polyclonal antibodies (avian) attached to uniform polystyrene particles	Gentian Cystatin C Calibrator Ref 1012 Lot 1405402: 7.70 mg/L	3.0%	ERM-DA471/IFCC
Gentian Cystatin C/Roche Cobas c502	Avignon/Montpellier	PETIA	Yes The entire measurement system is CE marked (Gentian)	Polyclonal antibodies (avian) attached to uniform polystyrene particles	Gentian Cystatin C Calibrator Ref 1012 Lot 1405402: 7.70 mg/L	3.0%	ERM-DA471/IFCC
Roche Tina-Quant Cystatin C Gen.2/Cobas c502	Avignon/Montpellier	PETIA	Yes (Roche)	Polyclonal antibodies (rabbit) coated latex particles	C.f.a.s. Cystatin C Ref 04 975 901 191 Lot 61296701:7.11 mg/L	0.2%	ERM-DA471/IFCC
Siemens (Siemens Healthcare Diagnostics) N Latex Cystatin C/BN ProSpec	Avignon/Lyon Lab 2	PENIA	Yes (Siemens)	Polyclonal antibodies (rabbit) coated latex particles	N Protein Standard UY Ref OQLV055 Lot 049865: 1.98 mg/L	5.0%	ERM-DA471/IFCC
Siemens Flex CYSC/Vista	Bichat/Sart-Tilman	PENIA	Yes (Siemens)	Polyclonal antibodies (rabbit) coated latex particles	PROT3 CAL Ref KC770C Lot 5GC081: 1.90 mg/L	2.7%	ERM-DA471/IFCC

[a] PETIA, particle-enhanced turbidimetric immunoassay; PENIA, particle-enhanced nephelometric immunoassay.

COMMUTABILITY ASSESSMENT

The commutability of the 4 frozen pools was evaluated by measuring CysC in the 4 frozen pools and in 30 unmodified fresh serum samples. The 4 pools and 30 individual serum samples were measured in triplicate in the same analytical sequence using the 8 different CysC assays. Statistical analysis of commutability was performed based on the pair-wise comparison of sets of CysC results using a Deming regression, followed by the calculation of the 95% prediction intervals. Conclusions regarding the commutability of the frozen pools were drawn based on the positions of their values with respect to the prediction interval according to the CLSI guideline EP30-A (formerly C53-A) *(13)*.

UNCERTAINTY CALCULATION

In line with the 1st Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine, the analytical performance of the assays at the clinical sample level was evaluated using the biological variation model *(14)* with quality performance specifications based on both the intra- (5%) and interindividual (13%) biological variation of CysC *(15)*. According to the performance specifications *(16)*, the minimum, desirable, and optimum biases of CysC assays should be <5.22%, 3.48%, and 1.74%, respectively, whereas the minimum, desirable, and optimum imprecisions should be <3.75%, 2.5% and 1.25%, respectively. The expanded uncertainty (U) of the CysC measurements for a clinical laboratory using an unbiased assay at the patient sample level should remain approximately within ±2.5%, ±5.0%, and ±7.5% for the optimum, desirable, and minimum quality levels, respectively (e.g., the goals for imprecision multiplied by a coverage factor of 2) according to the approach described by Pasqualetti et al. *(17)*.

The combined uncertainty of the CysC measurement was estimated according to the model proposed by Magnusson et al. *(18)* by combining the bias and imprecision data obtained by measuring the IDMS value assigned pools. The resulting combined standard uncertainty (u_C) was calculated as follows: $u_C = (u^2_{Bias} + u^2_R)^{0.5}$, where u_R was the random component of the uncertainty. The relative u_C (%) was reported as the mean of the daily means. The corresponding expanded uncertainty was obtained by multiplying the combined standard uncertainty by a coverage factor of 2 ($U_C = 2u_C$).

The standard uncertainty associated with the bias (u_{Bias}) was calculated by combining 3 elements: the bias corresponding to the difference between the obtained mean of the means (n = 18) and the IDMS value, the bias variability corresponding to the SD of the individual bias divided by the square root of the number of measurements, and the relative standard uncertainty associated with the certified value of the reference pool material *(19)*.

The standard uncertainty associated with the random effect (u_R) was obtained using the imprecision data (S_R) (n = 18). Imprecision was estimated according to the CLSI EP05-A3 guidelines *(20)*. A 2-way nested analysis of variance (ANOVA) was performed for each assay at each pool concentration, and the treating "site" and "days" were 2 factors. Three precision types were estimated: (*a*) the repeatability SD (S_r), (*b*) the within-laboratory precision SD (S_{Rw}), and (*c*) the reproducibility SD (S_R).

STATISTICAL ANALYSIS

Statistical analysis was performed using R 3.1.0 software (R Foundation for Statistical Computing, Vienna, Austria).

Results

COMMUTABILITY ASSESSMENT OF THE 4 FROZEN POOLS

Based on the Deming regression, all the pools were within the 95% prediction intervals and considered commutable in all 28-pairwise comparisons (see Supplemental Figs. S1–S28 in the Data Supplement that accompanies this article at http://www.clinchem.org/content/vol63/issue4), suggesting that these materials were appropriate for estimating the trueness of the assays.

IMPRECISION

The total imprecision (S_R) reached the minimum specification of 3.75% for all of the assays, except for DiaSys implemented on the Architect platform in pools 1, 2, and 3, Roche reagents on the Cobas system in pools 1 and 3, and Abbott reagents on the Architect system in pool 1. However, when considering only the intralaboratory imprecision, all assays reached the minimum criterion. Gentian implemented on the Architect platform produced better results than when it was implemented on the Cobas analyzer. The Abbott assay exhibited the best results with an intralaboratory imprecision of <1.25% at all concentrations. The 2 Architect platforms had an interlaboratory imprecision (S_R) that was 2-times higher than the intralaboratory imprecision (S_{Rw}) (Table 2).

BIAS AGAINST THE CANDIDATE RMP

The IDMS-assigned CysC concentrations and associated expanded uncertainties (k = 2) were 0.957 (0.036) mg/L (3.7%), 1.118 (0.041) mg/L (3.6%), 1.445 (0.027) mg/L (1.9%), and 1.962 (0.056) mg/L (2.9%) for pools 1, 2, 3, and 4, respectively.

The optimal specification for bias was reached with Siemens reagents on the BN ProSpec system and with DiaSys reagents on the Architect system in pools 2, 3, and 4, whereas a desirable goal for bias was reached for the 2 platform and reagent combinations in pool 1. On the Vista system, an optimal bias was reached in pools 2 and 4, and a

Table 2. Mean values, relative biases (%) vs IDMS, imprecision expressed as the relative SD% and the relative standard uncertainties for each contributing factor for the determination of CysC for the different pools and reagent–analyzer– calibrator combinations.[a]

	Abbott/ Architect	Diasys/ Architect	Diasys/ Cobas c502	Gentian/ Architect	Gentian/ Cobas c502	Roche/ Cobas c502	Siemens/BN ProSpec	Siemens/ Vista
Pool 1 (0.957 ± 0.036 mg/L)								
Mean, mg/L	1.15	0.94	0.93	0.98	0.96	1.04	0.98	0.94
Bias, %	20.1	−1.8	−3.0	2.3	0.1	8.3	2.3	−2.3
u_{Bias}, %	20.2	2.6	3.5	3.0	1.9	8.5	2.9	3.0
S_r, %	1.1	2.6	2.0	1.7	2.4	3.6	1.5	1.1
S_{Rw}, %	1.2	3.6	2.0	1.7	3.5	3.6	1.5	1.6
$S_R = u_R$, %	4.0	9.5	2.3	2.1	3.5	4.3	2.5	1.9
u_C (k = 1), %	17.3	9.9	4.3	3.6	4.0	9.0	3.8	3.6
U_C (k = 2), %	34.6	19.8	8.6	7.3	8.0	18.0	7.6	7.2
Pool 2 (1.118 ± 0.041 mg/L)								
Mean, mg/L	1.34	1.12	1.10	1.17	1.13	1.20	1.14	1.11
Bias, %	19.7	0.2	−1.3	4.4	1.4	7.2	1.7	−0.3
u_{Bias}, %	19.8	1.8	2.2	4.8	4.2	7.4	3.5	2.5
S_r, %	1.0	1.7	1.8	1.3	2.3	2.0	1.7	1.4
S_{Rw}, %	1.3	2.3	1.8	1.3	3.5	2.3	2.0	1.9
$S_R = u_R$, %	2.9	6.3	3.0	1.5	3.5	2.7	2.5	2.0
u_C (k = 1), %	16.8	6.5	3.7	4.8	4.2	7.4	3.5	2.7
U_C (k = 2), %	33.6	13.1	7.5	9.6	8.4	14.8	7.0	5.5
Pool 3 (1.45 ± 0.027 mg/L)								
Mean, mg/L	1.70	1.45	1.42	1.53	1.49	1.54	1.43	1.40
Bias, %	17.0	−0.2	−2.2	5.2	2.7	5.9	−1.1	−3.2
u_{Bias}, %	17.0	1	2.4	5.3	2.9	6.0	1.5	3.4
S_r, %	0.7	1.3	1.5	0.8	2.4	2.6	1.3	0.9
S_{Rw}, %	0.8	1.3	1.8	1.2	2.4	2.9	1.4	1.6
$S_R = u_R$, %	2.8	4.1	1.8	1.2	2.6	3.9	1.4	1.6
u_C (k = 1), %	14.8	4.2	3.0	5.2	3.9	6.9	2.0	3.8
U_C (k = 2), %	29.6	8.5	6.1	10.3	7.7	13.7	4.1	7.7
Pool 4 (1.962 ± 0.056 mg/L)								
Mean, mg/L	2.28	1.95	1.91	2.14	2.04	2.06	1.98	1.95
Bias (%)	16.3	−0.4	−2.7	8.9	3.9	5.2	0.9	−0.4
u_{Bias}, %	16.4	1.5	3.1	9.0	4.2	5.4	1.7	1.5
S_r, %	0.5	2.2	1.7	0.7	1.5	2.0	1.5	1.8
S_{Rw}, %	1.2	2.2	2.4	0.7	2.4	2.2	2.4	2.7
$S_R = u_R$, %	3.1	2.2	2.4	0.7	2.4	3.1	2.6	3.1
u_C (k = 1), %	14.4	2.7	4.0	8.3	4.7	6.0	3.1	3.5
U_C (k = 2), %	28.8	5.4	8.0	16.6	9.4	11.9	6.1	7.0

[a] The minimum, desirable, and optimum bias values should be <5.22%, 3.48%, and 1.74%, respectively. The minimum, desirable, and optimum imprecisions should be <3.75%, 2.5%, and 1.25%, respectively. The minimum, desirable, and optimum expanded combined uncertainties (U) should be <7.5%, 5.0%, and 2.5%, respectively.

desirable bias was reached in pools 1 and 3. DiaSys reagents on the Cobas platform achieved the desirable goals in all the pools. Gentian reagents on the Cobas system reached the optimal level in pools 1 and 2, the desirable level in pool 3, and the minimum level in pool 4. The same reagents on the Architect system reached the desirable level in pool 1 and the minimum level in pool 2, but did not achieve the minimum level in pools 3 and 4.

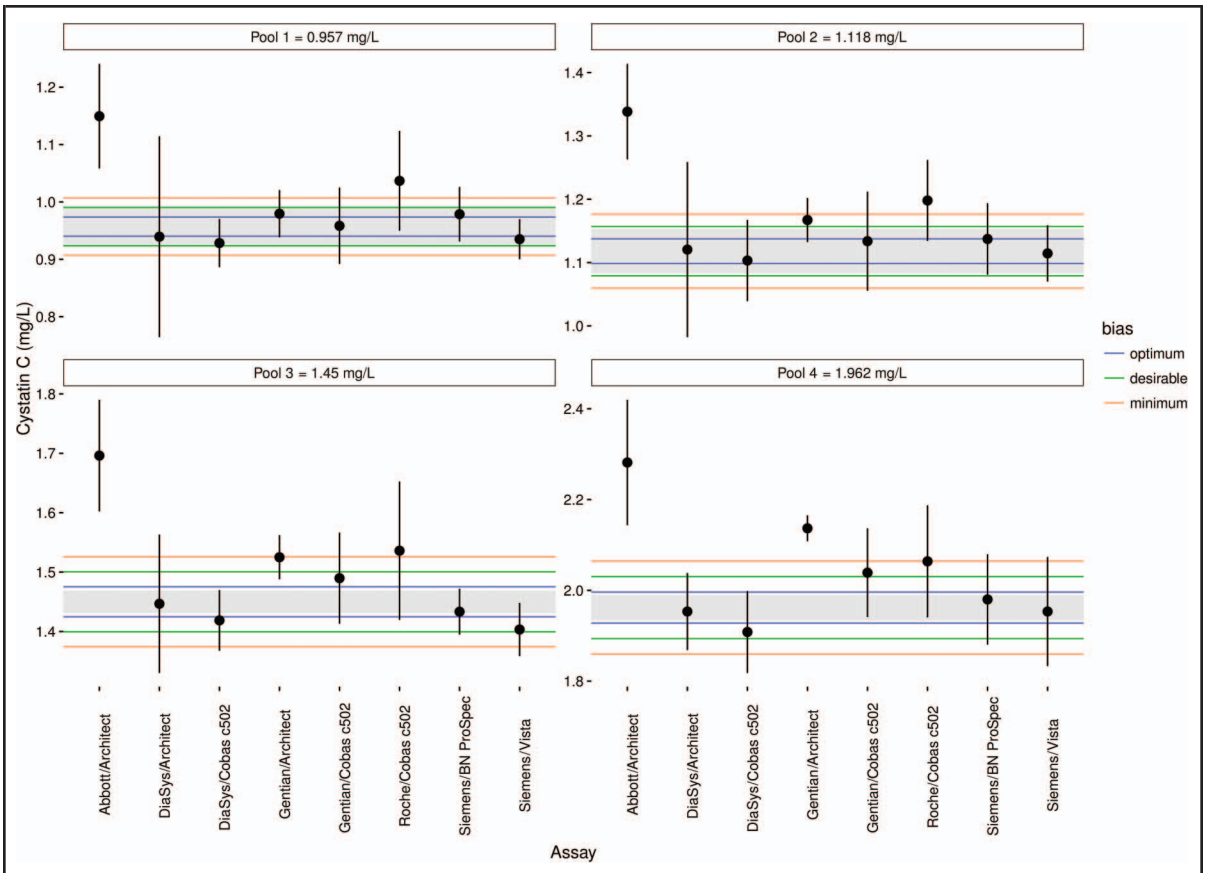

Fig. 1. Biases between the commercial assays and the IDMS target value for CysC.

The figure shows the reference material values and the areas of their uncertainty (grey zone), the mean (SD) of the results from the evaluated systems, and lines depicting the bias specifications (red, green, and blue lines correspond to the optimal, desirable, and minimum quality levels, respectively).

Abbott reagents on the Architect system (pools 1, 2, 3, and 4), and, to a lesser extent, Roche reagents on the Cobas system (pools 1, 2, and 3) did not reach the minimum criterion being biased from 16.3%–20.1% for Abbott reagents on the Architect system and from 5.2%–8.3% for Roche reagents on the Cobas system (Table 2, Fig. 1).

ESTIMATION OF THE COMBINED UNCERTAINTY

As shown in Table 2, the minimum acceptable relative expanded uncertainty was only obtained for a few assays: DiaSys reagents on the Cobas system (pools 2 and 3), DiaSys reagents on the Architect system (pool 4), Gentian reagents on the Architect system (pool 1), and Siemens reagents on the BN ProSpec (pools 2, 3, and 4) and Vista systems (pools 1, 2 and 4). To evaluate the relative parts of each component separately, the relative combined expanded uncertainty was further calculated, first using the intralaboratory imprecision and then without bias (see online Supplemental Table 2). By using the intralaboratory imprecision, the minimum acceptable results for the combined uncertainty were obtained for Siemens reagents on the BN ProSpec system (pool 1) and DiaSys reagents on the Architect system (pools 2 and 3). Furthermore, after correcting for bias, the relative expanded uncertainty ranged from 5.9%–19.4% and was below 10% for all combinations except the DiaSys reagents on the Architect system (pools 1 and 2). These results strongly supported a residual bias as the main source of uncertainty in most of the assays, except the DiaSys reagents on the Architect system, for which the interlaboratory component was predominant.

Discussion

In this study, we showed that although a secondary reference material has been introduced to align assays, several commercially available measurement procedures remain clinically unsatisfactory with respect to the combined expanded uncertainty because of unacceptable

bias. However, random variability is not a major problem for CysC assays.

Only Siemens reagents on the Siemens systems, and to a lesser extent the DiaSys reagents on the Cobas system, provided results that satisfied the minimum performance criterion calculated according to the intraindividual and interindividual biological variations. These results were obtained using candidate RMP assigned values to commutable pooled serum control materials with CysC concentrations ranging from 0.957–1.962 mg/L. These values correspond to stages 1–4 of chronic kidney disease in a wide variety of patients.

The uncertainty attributable to imprecision was in agreement with data we collected in 2008 *(10)*. The imprecision of the assays was not a problem except when reagents not provided by the instrument platform manufacturer were used. It should be noted that a clear between-laboratory effect was present in the poor combined uncertainty obtained for DiaSys reagents on the Architect platform; this effect was not observed using DiaSys reagents on the Cobas system. These results confirm the importance of considering all the components of a measuring system, including the platform, calibrator, reagents, and control materials *(21)*. Measuring systems from the same manufacturer, as a whole, were all CE marked. The reagents/calibrators/controls implemented on the Architect and Cobas c502 platforms were CE marked for Gentian, whereas the applications proposed by DiaSys were not CE marked and are intended to serve as guidelines only. Thus, the measured results must be validated by the clinical laboratories and assessed with caution. However, CE marking does not guarantee that the manufacturer has successfully transferred trueness.

It is logical to expect that with the availability of ERM-DA471 CysC results should be unbiased when compared to those from commutable pooled serum materials with a value assigned by the candidate RMP. Our results demonstrated that unacceptable bias remains for the clinical use of CysC measurements. Although some manufacturers have improved their calibration protocols to correct bias, others have not correctly implemented traceability to ERM-DA471. The Siemens measurement procedures (Siemens reagents on Siemens systems) evaluated here were the ERM-DA471/IFCC-traceable versions, and we observed a significant improvement in 2015 with a bias of $<3.2\%$ at all tested concentrations. This finding differed from the negative bias (ranging from -11 to -18%) we observed in our previous European study performed in 2008 *(10)*, and in the CAP study performed in 2014 *(8)*. Siemens restandardized its CysC assays against ERM-DA471/IFCC using a constant conversion factor of 1.11 over the entire measuring range in 2012 (for BN ProSpec systems) and 2013 (for Dimension Vista Intelligent Laboratory Systems) outside the US. However, the transition is still ongoing and was finalized in mid-2014 for most non-US countries only *(22)*. The continued availability of 2 reagent/calibrator kits for the Siemens systems' that have different calibrations (one traceable to the ERM-DA471/IFCC reference material and one not traceable) creates considerable confusion *(23)*. Indeed, Zhao et al. *(24)* demonstrated that the BN2 CysC results obtained in the US remained inaccurate 5 years after the introduction of ERM-DA 471.

Abbott reagents on the Architect platform exhibited positive bias ranging between 16.3% and 20.1% at all tested concentrations and this bias was not reduced compared to that obtained using the Abbott system in 2008 *(25)*. The data obtained in this evaluation clearly highlight some important issues in the calibrator value-assignment protocol for transferring trueness from higher-order references to the Abbott calibrator used for the CysC assay. To a lesser extent, the Roche reagents on the Cobas c502 system still exhibited clinically significant positive bias ranging from 5.2%–8.3%, which decreased with increasing concentrations. This result was comparable to that obtained in 2008 *(10)* before standardization, but inconsistent with those obtained in the CAP 2014 CysC survey, in which the Roche results demonstrated a high level of agreement using fresh-frozen samples with ERM-DA471/IFCC–traceable target values *(8)*. Similarly, Ebert et al. *(7)* demonstrated that the bias between the Roche and Siemens CysC assays was reduced from 7% with the Roche Gen1 to 0% with the Roche Gen2 standardized assay. Gentian reagents displayed increasing bias with increasing concentration on both the Architect and Cobas systems. The use of Roche systems by the CAP participants was not as strictly controlled as in our study; therefore, some assay "customization" by individual laboratories cannot be excluded. Such customization could explain the discrepancies between the results of our study and those of the CAP study. In addition, inconsistency in the assignment of values to the commercial calibrators by the manufacturer and the potential of between-lot (reagents and calibrator) variations cannot be excluded. Because ERM-DA471/IFCC consists of a 1-level calibrator with a high CysC concentration (5.48 mg/L), dilutions are required to prepare additional calibrators with lower CysC concentrations. It can be speculated that some assay manufacturers failed to identify an appropriate dilution buffer and/or used noncommutable dilutions and/or transfer calibrators.

At the patient level, the uncertainty budget combines the uncertainty of the reference material, the uncertainty of the commercial assay calibration, the system imprecision and the individual laboratory variability. Braga et al. recently recommended that 33% and 50% of the total uncertainty budget should be consumed by the uncertainty of the references and the manufacturer's calibration and value transfer protocol *(26)*. The relative expanded uncertainties of the IDMS-based targets of the

pools were close to that of the ERM-DA471/IFCC calibrator (U = 2.74%). The uncertainties of current state-of-the-art for absolute protein quantification by IDMS are not much lower than those reported in our study. However, IDMS-assigned values are supposed to be unbiased because IDMS is considered as a high-order measurement procedure. Diagnostic manufacturers are expected to implement suitable analytical systems (i.e., platforms, reagents, calibrators, and controls) that fulfill the established goals of the field [Joint Committee for Traceability in Laboratory Medicine (JCTLM), IFCC, European Federation of Clinical Chemistry and Laboratory Medicine (EFLM)] *(21)*. In vitro diagnostics (IVD) manufacturers should provide a calibrator level uncertainty that includes the uncertainty associated with higher levels of the selected metrological traceability chain *(21, 26)*. For example, the uncertainty declared by Roche for its commercial calibrator (C.f.a.s. CysC) is 0.2%, which is lower than that declared for the reference material ERM-DA471 (2.74%), which was used by the same company to transfer the measurement trueness. Clearly, this value corresponds to uncertainty without including the higher-order ERM uncertainty. The role of clinical laboratories is to verify consistency with the performance claimed by a measurement system manufacturer through daily quality operations performed according to the manufacturer's instructions and structured external quality assessment programs that meet metrological criteria *(27)*.

The 2012 Kidney Disease: Improving Global Outcomes recommendations suggest measuring the $eGFR_{Crea,CysC}$ in adults with eGFR based on creatinine ($eGFR_{Crea}$) values in the range of 45–59 $mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}$ who do not have markers of kidney damage *(3)*. Consequently, the measurement uncertainty is especially important when low concentrations of CysC might be present because large variations in this range can dramatically impact the GFR estimation *(28, 29)*.

The strengths of this study are that, for the first time, 8 CysC assays that claim to be standardized to ERM-DA471/IFCC reference material were evaluated using commutable pools with concentrations assigned by a candidate RMP. The limitation of this study is that measurements were conducted in a small subset of clinical laboratories that are active in chronic kidney disease investigations (n = 7; 2 laboratories for each measuring system). In comparison, a much larger number of laboratories (n = 141) were involved in the Eckfeldt study *(8)*. The advantage of the latter study is that it could better mimic "real-life" conditions. In contrast, expert laboratories are probably more dedicated to evaluating the performances of an assay.

In conclusion, among the CE marked IVD assays, only Siemens reagents on the Siemens systems met the desirable specifications for bias. IVD manufacturers should improve the metrological traceability of their analytical systems to the available higher-order references *(21)*. With few exceptions, the equivalence of CysC results among the commercially available measuring systems has not been achieved despite the availability of the ERM-DA471/IFCC reference material.

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