Importance of Cystatin C Assay Standardization

To the Editor:

Cystatin C is an alternative blood biomarker of kidney function (1, 2). Relatively little effort has been devoted to standardization of cystatin C measurement until recently, when the IFCC formed a working group to produce an international certified cystatin C reference material until recently, when the IFCC formed a working group to produce an international certified cystatin C reference material, which was released in June 2010. In the current study, we evaluated a new cystatin C particle-enhanced turbidimetric assay (PETIA) (3) (Gentian AS) that is traceable to this certified cystatin C reference material (3) and can be routinely run on a chemistry autoanalyzer, therefore potentially increasing availability and decreasing costs (4).

For assay validation, waste patient serum that had been stored for no more than 7 days at 4 °C (n = 102) was obtained from the Mayo Clinic Central Clinical Laboratory. We also studied biobanked samples for which cystatin C had been measured by particle-enhanced nephelometric assay (PENIA) (1) (Gentian AS) that is traceable to certified cystatin C reference material (5) and that had subsequently been stored at −70 °C without any intervening freeze–thaw cycles. The study was approved by the Mayo Clinic Institutional Review Board.

The Gentian cystatin C PETIA was deployed on a cobas 6000/501c analyzer (Roche Diagnostics) according to the manufacturer’s instructions and with reagent kits supplied by Atlantic Diagnostics. Cystatin C was also measured with the Siemens (previously Dade Behring) PENIA, as previously described (3). The ERM-DA471/IFCC reference material was produced by the Institute for Reference Materials and Measurements and was obtained from Analytical Reference Material International (3).

The PENIA and PETIA methods were compared by Passing–Bablok regression analysis; Bland–Altman plots were used for analyzing the difference between these 2 methods. Results were analyzed with the statistical analysis programs Analyse-it® (version 2.12; Analyse-it Software), JMP® (version 8; SAS Institute, www.sas.com), and Microsoft Excel (version 2003; Microsoft Corporation).

PETIA measurements of cystatin C produced imprecision estimates (CVs) of 0.65% to 1.3% at cystatin C concentrations between 0.98 and 1.88 mg/L. The lower limit of quantification was established at 0.35 mg/L with a CV of <2% by measuring 3 serum samples with signals just above those of the lowest calibrator 5 times each. Linearity studies of serum and plasma samples and the international standard diluted with water yielded percentages of the measured cystatin C concentration with respect to the expected concentration of 90%–105% over a signal interval of 0.33–5.97 mg/L, thereby setting the upper end of the analytical measurement interval at 6 mg/L. The response was linear and parallel when high-signal serum (1.87 mg/L) and plasma (3.00 mg/L) samples and the certified reference material (5.97 mg/L) were diluted up to 8-fold. To further assess performance, we carried out a study in which we mixed a high-signal patient serum sample (3.05 mg/L) and 3 low-signal serum samples (0.99–1.29 mg/L) and demonstrated recovery rates of 100%–105% of the predicted value.

是非標準的縮寫：PETIA, particle-enhanced turbidimetric assay; PENIA, particle-enhanced nephelometric assay; eGFR, estimated glomerular filtration rate.
When we reanalyzed a subset of samples from the assay-validation cohort (n = 40) with the PENIA currently deployed in the Mayo Clinic laboratory, we observed an unexpected 25% positive difference for the PETIA method (95% CI, 15%–36%). To further examine this difference between the 2 methods, we reanalyzed biobanked samples from a reference value study of the PENIA carried out at the Mayo Clinic in 2000. Interestingly, the current PENIA results were 19% lower across the measurement interval compared with values obtained in 2000 with the same PENIA platform (Fig. 1). The difference was in a direction that could account for much of the discrepancy observed between the current PENIA and PETIA results (within the 95% CI). Other laboratories have also recently reported a similar drift in the PENIA assay over time (5). When we combined the data for 142 samples run on both platforms, we observed 23% higher values overall for the PETIA method.

To evaluate this interassay difference further, we reconstituted the certified IFCC cystatin C reference material according to the manufacturer’s instructions and then diluted it with 3 volumes of normal saline to obtain a target concentration of 1.38 mg/L, which is well within the expected clinical interval. The Gentian PETIA yielded a cystatin C concentration of 1.35 mg/L (98% of target), with the corresponding PENIA result being 1.10 mg/L (80% of target). Thus, the PETIA, but not the PENIA, yields the expected results for this international reference material.

In conclusion, PETIA can accurately measure cystatin C on a chemistry autoanalyzer. The PETIA had 23% higher values compared with the PENIA currently used in the Renal Function Laboratory at the Mayo Clinic. Using a recently published equation for the estimated glomerular filtration rate (/4/), we find that an increase in the cystatin C concentration from 1.00 mg/L to 1.23 mg/L changes the calculated eGFR from 77 mL·min⁻¹·(1.73 m²)⁻¹ to 60 mL·min⁻¹·(1.73 m²)⁻¹, whereas a shift from 2.00 mg/L to 2.46 mg/L changes the eGFR from 34 mL·min⁻¹·(1.73 m²)⁻¹ to 26 mL·min⁻¹·(1.73 m²)⁻¹. Furthermore, banked samples that had been analyzed by the PENIA in 2000 yielded results that were 19% lower when they were rerun by the same PENIA in 2010. Therefore, this study highlights the importance of standardization if cystatin C is to be more widely used to estimate the GFR and place patients in the correct chronic kidney disease stage.

**Fig. 1.** Cystatin C PENIA results for biobanked samples analyzed in 2000 and again in 2010. Bland–Altman plot. Indicated are the mean percentage difference and the 95% CI.

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\[(2000 PENIA value) \times (2010 PENIA value)\]/[(mean of 2000 and 2010 PENIA values) \times 100%].

**Author Contributions:** All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

**References**


**Nikolay V. Voskoboev**

**Timothy S. Larson**

**Andrew D. Rule**

**John C. Lieske**
Impact of Implementation of the High-Sensitivity Cardiac Troponin T Assay in a University Hospital Setting

To the Editor:

The performance of the high-sensitivity cardiac troponin T assay (hs-cTnT)\(^1\) has been evaluated in a multicenter study (1). Effective July 2009, we replaced the fourth-generation troponin T assay (cTnT) with the hs-cTnT assay in clinical practice. This study audits the impact of this implementation.

The hs-cTnT, implemented on the cobas e 411 platform (Roche Diagnostics), fully replaced the cTnT performed on the Elecsys 2010 analyzer [cutoff, 30 pg/mL—based on actual assay performance (10% CV concentration)]. We obtained a detection limit of 5 pg/mL, a 99th percentile of 15 pg/mL, limited comparability with the cTnT at concentrations <100 pg/mL (on average, a 30-pg/mL cTnT concentration yielded a value of approxi- mately 65 pg/mL with the hs-cTnT) and mean CVs of 9.1% for the cTnT (at 39 pg/mL) and 8.5% for the hs-cTnT (at 17 pg/mL). We retrieved hs-cTnT results for the first 3 months after implementation (July 16 to October 15, 2009) and cTnT results for the same period 1 year previously. Results were dichotomized as positive or negative with respect to cutoffs. Among troponin-positive patients with at least 2 results during their examination, we divided marker-release curves on the basis of typical or atypical kinetics. We defined as “typical” an increasing or decreasing pattern showing a troponin change between 2 consecutive samples exceeding +46% for increasing troponin results and −32% for decreasing results. Otherwise, the troponin pattern was considered “atypical.” For definition of these percentage changes, we referred to the short-term biological variation for troponin I (2). We are aware, however, that the 2 cardiac troponins may have different biological kinetics in blood, so their biological variation may be different.

In the evaluated period, 2287 hs-cTnT tests were performed during 1371 examinations of 1137 patients. Correspondingly, 2170 cTnT tests were performed during 1409 examinations of 1205 patients. After hs-cTnT implementation, a 5.4% increase in the hospital-wide test volume was recorded, despite a slight decrease in the number of admitted patients and examinations. The mean (SD) number of troponin tests per examination was 1.54 (1.0) before and 1.67 (1.1) after hs-cTnT implementation (P < 0.0001), with a single test ordered in 67.5% and 60.2% of examinations, respectively. The distribution of troponin orders and positive-test rates in different wards is shown in Table 1. A positive result was found in 31.7% of cTnT tests and in 58.7% of hs-cTnT tests (relative difference, +85%), corresponding to 25.3% and 51.6% positive examinations, respectively (P < 0.0001). Of all the hs-cTnT positive results, 64% fell in the 16–65 pg/mL interval, previously negative with the cTnT. In the emergency department after hs-cTnT implementation, the number of hospitalized patients with positive troponin results increased from 158 to 292 (+85%), but the rate of admission in intensive care and non–intensive care departments was unchanged (P = 0.108). In the same periods, 16 cTnT-positive patients (8.5%) and 109 hs-cTnT–positive patients (26.6%) were discharged. Of these discharged patients, 1 cTnT–positive patient and 13 hs-cTnT–positive patients were readmitted to the emergency department in the subsequent 2 months (P = 0.804, between the 2 assays).

We audited 458 cTnT and 546 hs-cTnT curves, of which 39.1% and 69.0%, respectively, had at least 1 positive result (P < 0.0001). The difference in the percentage of positive curves displaying a typical marker release was not significant (17.2% for the cTnT vs 20.5% for the hs-cTnT, P = 0.32). A higher absolute number of typical positive curves was observed after hs-cTnT implementation (from 79 to 112). This increased ability to detect events involving acute marker release was fully explained by the number of typically positive curves in which the hs-cTnT result never exceeded 65 pg/mL (n = 38).

The replacement of the cTnT with the hs-cTnT markedly increased the rate of positive tests. A similar outcome was previously described for a contemporary sensitive troponin I assay (3). What is unique in our experience is the magnitude of the increase in positive results after hs-cTnT introduction, which was based on imple-