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 (ERM®-DA471/IFCC), which was released in June 2010. In the current study, we evaluated a new cystatin C particle-enhanced turbidimetric assay (PETIA)1 (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) in 2000 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.

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 4 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

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1 Nonstandard abbreviations: PETIA, particle-enhanced turbidimetric assay; PENIA, particle-enhanced nephelometric assay; eGFR, estimated glomerular filtration rate.
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.

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**References**


