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Automation and Analytical Techniques

Initial evaluation of cystatin C measurement by particle-enhanced immunonephelometry on the Behring nephelometer systems (BNA, BN II)

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Serum cystatin C has been suggested as a new marker of glomerular filtration rate (GFR). We describe a fully automated and rapid particle-enhanced nephelometric immunoassay (PENIA) for measuring serum cystatin C on the Behring nephelometer systems (BNA, BN II). Each sample is analyzed in 6 min with as many as 75 samples per batch. The assay covers the range 0.23–7.25 mg/L, up to seven times the upper limit of normal. The intra- and interassay imprecision are <3.3% and <4.5%, respectively. There is absolute linearity across the assay range (r^2 = 0.997), with analytical recovery by cystatin C addition between 95% and 109% (mean 102%). Hemoglobin (≤8.0 g/L), bilirubin (≤488 μL), triglycerides (≤23 mmol/L), rheumatoid factor (≤2000 kIU/L), and myeloma paraprotein (≤41 g/L) do not interfere with the assay. This assay agreed well with an in-house particle-enhanced turbidimetric immunoassay (PETIA) (mean difference = 1.73 ± 2.10) and a commercial PETIA (mean difference = 1.13 ± 0.86). This is a new assay by which cystatin C may be effectively used as a marker of GFR estimation.

INDEXING TERMS: kidney function • immunoassay • glomerular filtration rate

Cystatin C is a nonglycosylated, low-molecular-mass (13 kDa) basic protein that is a member of the cystatin superfamily of cysteine protease inhibitors *[1–3]*. It consists of 120 amino acids and is produced by all nucleated cells, and even in inflammatory conditions the production rate is unaltered *[4, 5]*. Structural analysis of the cystatin C gene and its promoter has shown that the gene is of the housekeeping type, which is compatible with a stable production rate by most cells *[6]*. The low molecular mass of cystatin C and its high pI allow it to be freely filtered by the glomerular membrane. The serum concentration of this protein has been shown to correlate with the glomerular filtration rate (GFR) of the individual and, in combination with its stable production rate, suggests that cystatin C may be potentially a new marker of GFR *[4, 5, 7]*.[3]

Several other low-molecular-mass proteins, β_2-microglobulin, retinol-binding protein, and α_1-microglobulin (protein HC) have been investigated for their utility in monitoring GFR *[5, 8]*. None of these has proven useful, mainly because of the influence of nonrenal factors on their circulating concentrations; for example, infection, dietary factors, and liver disease may vary their production rate *[5, 7]*.

Creatinine and urea are more commonly used for the clinical assessment of GFR but they too have a range of nonrenal factors influencing their production, for example, muscle mass and protein intake, and for creatinine there are several well-reported difficulties concerning its analysis *[9, 10]*. An alternative to creatinine is needed that is analytically more reliable and as, or more, clinically reliable, i.e., a more sensitive and specific marker of nephron loss.

In previous years cystatin C measurement in serum has been suggested to correlate with GFR *[4, 5, 7]*. Previous investigations have confirmed that the serum concentration of cystatin C is at least as good an indicator of GFR as the serum concentration of creatinine *[4, 5, 7]*. For the

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[3] Nonstandard abbreviations: GFR, glomerular filtration rate; PENIA, particle-enhanced nephelometric immunoassay; CI, confidence interval; and PETIA, particle-enhanced turbidimetric immunoassay.

introduction of this marker into clinical use a rapid and automated method is required.

The cystatin C concentration in biological fluids is low, making high demands on the analytical sensitivity and specificity. In 1979, Löfberg and Grubb *[11]* developed the first enzyme immunoassay for quantifying cystatin C in human biological fluids and later recommended this as a kidney function test *[5]*. By present standards, the assay was time consuming and had a poor detection limit (see Table 1). Subsequently, simpler and more-sensitive radio-, fluorescence, and various enzyme immunoassays were developed to improve analytical reliability of the methods. In 1993, Pergande and Jung developed a sandwich enzyme immunoassay for determining cystatin C in serum by using commercially available antibodies *[17]*, but the assay time was still far from ideal for routine processing, especially urgent requests. Latex immunoassay is another nonisotopic method based on direct agglutination by a protein of latex particles on which a specific antibody has been conjugated. It is a homogeneous method that can be easily automated. One assay based on latex particle agglutination was the particle-counting immunoassay method used by Bernard et al. *[18]*, although no further detail is specifically given on cystatin C measurement. In 1994–95, two fully automated latex particle-enhanced turbidimetry assays for cystatin C *[19, 20]* were developed. These assays are both rapid, automated methods for measuring cystatin C.

Here we describe the evaluation of a rapid automated method for determining serum and plasma concentrations of cystatin C on the basis of particle-enhanced nephelometry. This method has been compared with the two turbidimetric methods in a three-way method and calibrator comparison.

Materials and Methods

A Behring nephelometer 100 system analyzer (BNA) was used in the evaluation of a cystatin C assay developed by Behringwerke Diagnostica, Marburg, Germany. The light source is an infrared high-performance light-emitting diode (840 nm). The signal change between 10-s and 6-min time points was monitored in a reusable cuvette within a fixed cuvette rotor segment. The reagents used were supplement A (to prevent nonspecific interactions, e.g., rheumatoid factor) and supplement B (detergent to enhance stability), both in liquid form; latex particle reagent, calibrator, and control were all supplied in lyophilized form. The calibrator was purified cystatin C from human urine.

There were two assays available for comparison with the proposed method. First was an in-house latex particle-enhanced immunoturbidimetry method performed on a Monarch 2000 centrifugal analyzer (Instrumentation Laboratory, Warrington, UK) operating at 37 °C. A tungsten lamp is used as the light source with the wavelength being produced by a scanning monochromator. Absorbance is monitored at 340 nm in a disposable cuvette rotor with a pathlength of 0.74 cm. The latex particles were prepared as described by Newman et al. *[19]* with rabbit anti-human cystatin C antiserum (Dakopatts, Copenhagen, Denmark; code no. A451) and 80-nm diameter chloromethyl styrene particles (Bangs Labs., Indianapolis, IN) *[21]*. The calibrator was purified recombinant cystatin C (a gift from A. Grubb, Lund, Sweden) prepared according to Abrahamson et al. *[22]*.

Second was the commercially available latex particle-enhanced turbidimetric immunoassay (PETIA) from Dakopatts (code no. 0071) performed on a Cobas Bio instrument (F. Hoffmann-La Roche, Basel, Switzerland), a single unit self-contained centrifugal analyzer operating at 37 °C. The light source is a high-intensity xenon flash in combination with a holographically inscribed grating monochromator. The change in absorbance at 340 nm was measured in a disposable cuvette rotor. This assay involved 38-nm carboxylate-modified latex particles obtained from Duke Scientific Corp., Palo Alto, CA, to which

Table 1. Comparison of immunoassays for determining cystatin C in serum.

Method	Detection limit, μg/L	CV, %[a]	Procedure duration, h	Reference intervals, mean ± SD (and range), mg/L	No. of subjects	Ref.
RID	300	11	~38	1.3 ± 0.26 (0.72–1.7)	46	*11*
EIA	30	10–12	~16	1.1 ± 0.42 (0.63–2.5)	30	*11*
RIA	1.3	n.d.	16–21	0.96 ± 0.20 (0.6–1.7)	100	*12*
FIA	~1	n.d.	1 or 3	n.d.		*13*
EIA	n.d.	n.d.	~4.5	1.10 ± 0.15	20	*14*
EIA	1.9	4–5	~5	0.75 ± 0.65, ≤20 years	85	*15*
				1.34 ± 0.95, >20 years	189	
EIA	0.195	4–8	~1.5	1.25 ± 0.22 (0.86–1.7)	50	*16*
EIA	0.9	3–9	2	1.78 ± 0.26, female	33	*17*
				2.14 ± 0.31, male		
PETIA	150	2.0–3.2	7 mins	(0.61–1.21)	27	*20*
PETIA	27	3–5	5 mins	<1.25		*19*
PENIA	170	3–5	6 mins	(0.64–1.04)	30	Our method

[a] Range of intra- and interassay variations.
RID, radial immunodiffusion; EIA, enzyme immunoassay; n.d., not done.

was conjugated the same rabbit anti-human cystatin C antiserum as above. The calibrator was purified human cystatin C assigned with recombinant cystatin C *[22]*.

Serum creatinine was measured on samples used in the method comparison with the fixed-interval Jaffe method on the Monarch.

Final assay procedure. The following optimal assay protocol for measuring cystatin C was used in all experiments. All dilutions are made with on-board diluent. The assay is performed at room temperature with a six-point calibration curve (Fig. 1) covering the range 0.23–7.25 mg/L (produced with an initial lyophilized calibrator reconstituted with water). The calibrator is sampled three times into predilution cups, resulting in seven dilutions. All but the 1:10 dilution are used in the calibration curve. Samples are prediluted to 1:100, in two stages, before being analyzed. Fig. 2 shows how the neat sample is diluted before being pipetted into a cuvette simultaneously with 10 μL of combined supplement reagent (0.5 mL of reagent B is added to each bottle of reagent A), followed by 40 μL of particle reagent. Each calibrator dilution is pipetted in the same way. The contents are mixed, with readings taken at 10 and 360 s. The change in the signal is converted into mg/L. A sample can be measured in 6 min, with further results every 8 s.

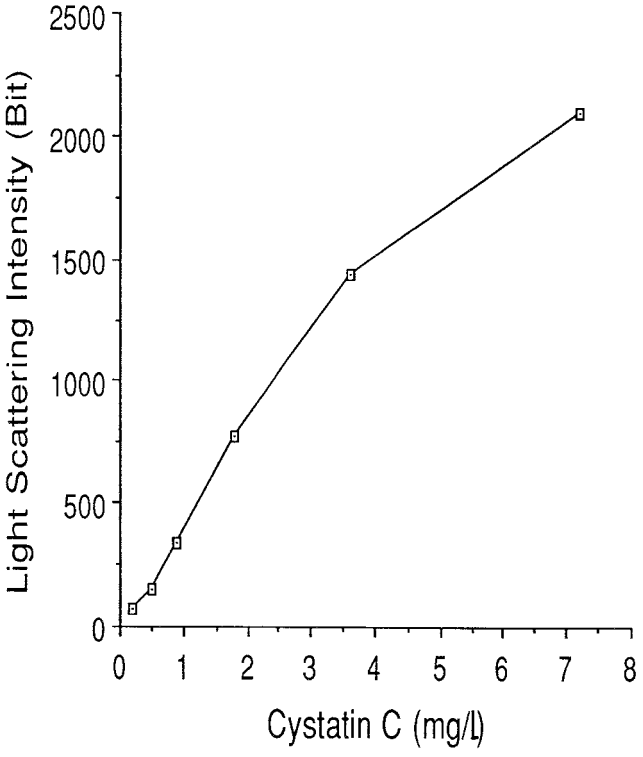

Fig. 1. Calibration curve for determination of cystatin C.

Lyophilized calibrator (1.45 mg/L) is reconstituted with water, then automatically diluted to produce a six-point calibration curve covering the range 0.23–7.25 mg/L.

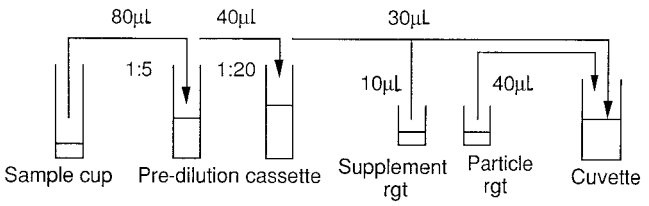

Fig. 2. Representation of the protocol of the cystatin C assay.

Each sample is diluted to 1:100 in two stages: (*a*) 80 μL of neat sample is diluted to 1:5; (*b*) 40 μL of the 1:5 dilution is further diluted to 1:20. Then 30 μL of the 1:100 dilution, together with 10 μL of the supplement reagent, is transferred to the cuvette, followed by 40 μL of the particle reagent and mixing of the components. Signal output is read at 10 s and 6 min with the change in signal calculated in mg/L.

ASSAY VALIDATION

Imprecision. The intraassay precision was assessed by using 20 replicate analyses of the Behring control (target value 0.99 mg/L) and three serum pools at approximately 1.0, 2.5, and 6.5 mg/L. The interassay precision was assessed by analyzing the control and serum pools across 28 different runs.

Linearity. Ten serum samples with high creatinine values were diluted (1+9, 2+8. . . . 9+1) in isotonic (9 g/L) saline to produce cystatin C values between 10% and 90% of the undiluted sample to assess linearity.

Analytical recovery. Analytical recovery was assessed with two 450-μL aliquots of 10 different serum samples by using two 50-μL supplemented cystatin C concentrations (0.52 + 0.93 mg/L). The percentage ratio between the measured and added concentrations of cystatin C for each sample was calculated.

Analyte stability. Twenty serum samples were obtained and analyzed within 8 h of their collection. These samples were aliquoted and stored at various temperatures: room temperature for 2 days, 4 °C for 1 week, −20 °C for at least 1 month, and −20 °C with 10 freeze/thaw cycles before analysis.

Plasma vs serum. The effect of anticoagulants was assessed by collecting blood from 12 healthy subjects into plain, heparin, and EDTA Vacutainer Tubes (Becton Dickinson, Franklin Lakes, NJ). The appropriate serum and plasma fractions were assayed for cystatin C. A further experiment with 19 matched serum and sodium citrate anticoagulated plasma samples were also assayed for cystatin C.

Interferences. The potential interferences of myeloma paraproteins (7–41 g/L), rheumatoid factor (98.5–2000 kIU/L), hemoglobin (1–8 g/L), bilirubin (38–488 μmol/L), and lipids (5.33–23.13 mmol/L) was assessed by assaying 10 patient samples of each increased interferent respectively and comparing any deviation from true linearity. A dilution procedure was carried out in which each sample was diluted (1+9, 2+8. . . 9+1, 10+0) with pooled patient serum containing no potential interferences.

Method comparison. A total of 120 patient samples was assayed in duplicate for cystatin C and for creatinine with the methods described. Each patient sample was obtained from the routine hospital laboratory and chosen on the basis of creatinine values.

Statistical analysis. Regression analyses were performed with the "Astute" statistical package (Diagnostic Development Unit; University of Leeds, Leeds, UK), as were the methods of Passing and Bablok *[23]* and Bland and Altman *[24]* for method comparison. The paired *t*-tests were performed with Statview® Abacus Concepts (Berkeley, CA) for Macintosh computers.

Results

Calibration curve. The calibration curve (Fig. 1) covers the range 0.23–7.25 mg/L over 6 calibration points.

Imprecision. Imprecision (CV) was <3.3% (intraassay) and <4.5% (interassay) across the assay range (Table 2).

Linearity. The results obtained (x) did not differ significantly from those expected (y) with the regression analysis equation $y = 0.18 + 0.94x$ ($r^2 = 0.997$, n = 100), indicating no lack of parallelism.

Analytical recovery. The average analytical recovery of cystatin C for each added concentration (0.52 and 0.93 mg/L) was 95% ± 2.2% (1SD) and 109% ± 0.03% (1SD), respectively.

Analyte stability. Although there was a 8% decrease in value with a paired *t*-test ($P < 0.05$), there was no significant difference between fresh samples and those measured after 2 days at room temperature, 1 week at 4 °C, or 1 week at −20 °C; cystatin C was thus considered stable at all temperatures over these time periods. However, there was a significant difference ($P < 0.05$) after 2 months at −20 °C, but the actual change in values was <0.14 mg/L with no trend across the time period. After 10 freeze/thaw cycles over 57 days there was a 15% decrease in value. A paired *t*-test was calculated ($P < 0.05$) and showed statistical significance but no trend with time; the greatest mean difference was 0.16 mg/L.

Table 2. Analytical imprecision of the cystatin C assay.

	Cystatin C, mg/L			
	Behring control	Low	Medium	High
Intraassay				
n	20	20	20	20
Mean	1.07	0.99	2.52	6.36
SD	0.03	0.03	0.06	0.13
CV, %	3.2	2.9	2.5	2.0
Interassay				
n	28	28	28	28
Mean	1.11	0.98	2.58	6.49
SD	0.04	0.04	0.11	0.29
CV, %	3.2	3.6	4.2	4.4

Plasma vs serum. There was no significant difference between EDTA and lithium heparin plasma cystatin C values. However, there was a statistically significant ($P < 0.05$) but small (3%) difference between the serum and plasma cystatin C, EDTA plasma having a bigger significant difference than lithium heparin plasma values. A comparison between 19 matched serum and sodium citrate plasma samples showed no significant difference after correction for sample dilution due to the volume of sodium citrate anticoagulant. The 12 normal serum samples gave a cystatin C concentration range of 0.60–1.45 mg/L.

Interference tests. Patient samples with hemoglobin concentrations ≤8 g/L did not interfere with the assay. The greatest deviation was 2.4% from the mean. Icteric patient samples with up to 488 μmol/L of bilirubin did not interfere, with the greatest deviation being 3.2% from the mean. Samples with increased triglyceride showed no interference with triglyceride as high as 23 mmol/L. The greatest deviation from the mean was 3.7%. Samples containing increased rheumatoid factor concentrations (98.5–2000 kIU/L) showed no significant interference with the assay, the greatest deviation being 5.1% from the mean. Samples with various myeloma types were investigated for interference; none could be found in samples containing up to 41 g/L paraprotein, with the greatest deviation of 5.0% from the mean.

Method comparison. Samples (120) were measured in duplicate in a three-way comparison with this cystatin C method (Behring nephelometer system), an in-house cystatin C method (Monarch 2000), and a commercial cystatin C kit from Dakopatts (Cobas Bio, Roche). Regression analysis (Passing and Bablok) were carried out on each comparison (Fig. 3). Regression for the particle-enhanced nephelometric immunoassay (PENIA) = −0.15 + 0.77 × Dakopatts [intercept 95% confidence interval (CI) = −0.21 to −0.12; slope 95% CI = 0.75 to 0.79] and 0.47 + 0.54 × in-house (intercept 95% CI = 0.44 to 0.47; slope CI = 0.54 to 0.55). Using the Bland and Altman method as a predictor of scatter, the mean difference between the PENIA and the in-house particle-enhanced turbidimetric immunoassay (PETIA) was 1.73 ± 2.10 mg/L and between the PENIA and Dakopatts PETIA was 1.13 ± 0.86 mg/L. For completeness, the agreement between the Dakopatts and in-house PETIA methods was established: in-house = −1.14 + 1.42 × Dakopatts (intercept 95% CI = −1.22 to −1.10; slope 95% CI = 1.39 to 1.45) and the mean difference was −0.50 ± 1.48 mg/L. Outliers 1 and 2 represent two samples that show good precision between duplicates in all methods but do not correlate well only in the Dakopatts assay. The only other information known about these two samples was their creatinine values, 143

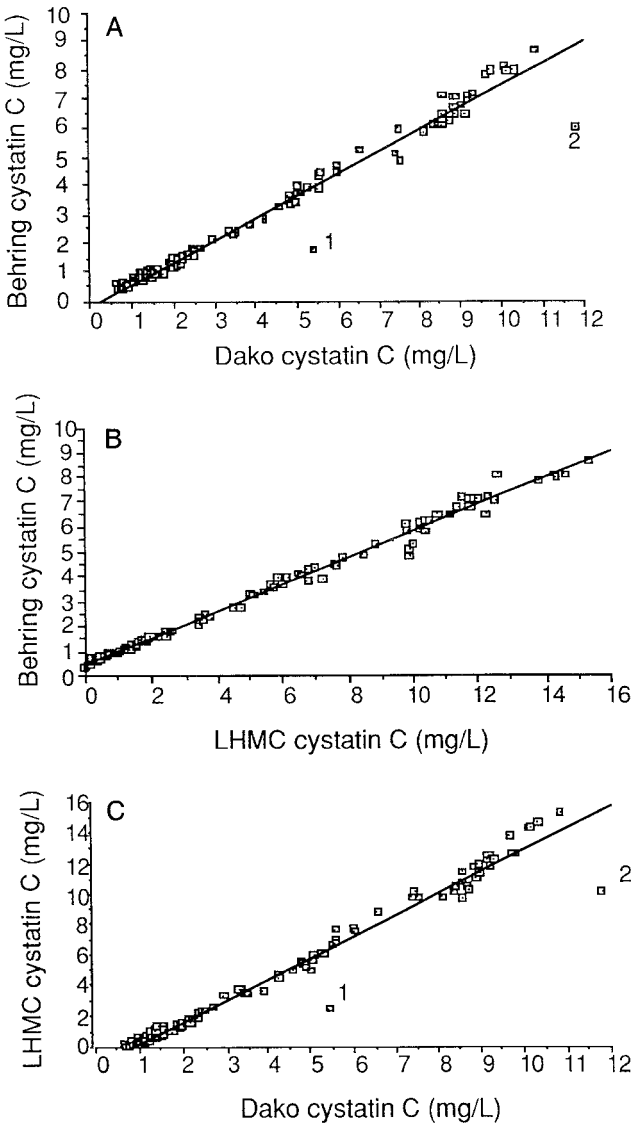

Fig. 3. Methods comparison between three particle-enhanced cystatin C immunoassays.

Passing and Bablok regression analyses were performed for each method comparison. (*A*) Dakopatts (PETIA) against Behring (PENIA); $y = 0.15 + 0.76x$, n = 120, $r = 0.97$. (*B*) In-house (PETIA) against Behring (PENIA); $y = 0.47 + 0.54x$, n = 120, $r = 0.99$. (*C*) Dakopatts (PETIA) against in-house (PETIA); $y = 1.13 + 1.40x$, n = 120, $r = 0.97$. Each graph shows the line of fit. Samples *1* and *2* are two outliers.

μmol/L and 444 μmol/L, respectively. Overall there is excellent correlation between the three methods. There were, however, differences between the slopes and intercepts as shown above; much of this can be explained by calibrator differences (see below). Fig. 4 shows the precision profile of each method. Although all three methods show excellent performance, the Behring assay shows better precision at <2.0 mg/L.

Calibrators. The three calibrators were from different sources and had different assigned cystatin C values (Table 3). Each calibrator was compared by measurement

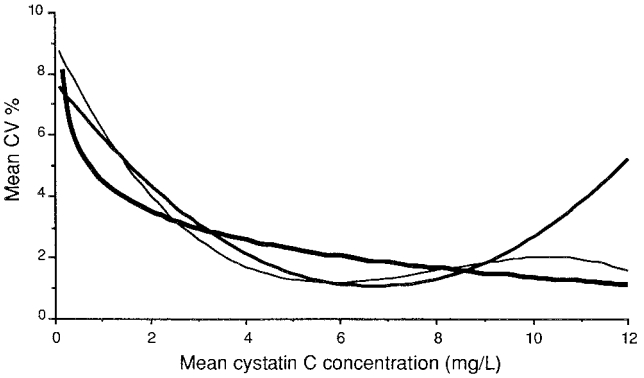

Fig. 4. Representation of precision profiles of the three methods.

PENIA (*thickest line*), Dakopatts PETIA (*thinnest line*), and in-house PETIA (*in-between line*) show the mean cystatin C concentrations against their mean percentage CV.

with each assay for cystatin C. Taking the value of the Behring calibrator as 100%, the calibrators from the Dakopatts and in-house methods as measured on the Behring nephelometer system were recalculated as a percentage of their respective assigned values. The Dakopatts calibrator was on average 70% of its assigned value, whereas the in-house calibrator was on average only 50% of its assigned value. When recalibrated, the slopes of the in-house assay against the Behring assay changed from 0.54 to 1.09, the Dakopatts assay against the Behring assay changed from 0.77 to 1.10, and the Dakopatts assay against the in-house assay changed from 1.42 to 1.01, thereby identifying the calibrators as the major source of the slope differences. Although the intercept between the new PENIA method and the Dakopatts method showed no difference, there was a 0.5 mg/L difference in the intercept between comparisons with our in-house method and both other methods. The in-house method has calibrator material contained in a horse serum matrix along with a zero calibrator, unlike the PENIA and Dakopatts methods, whose lowest calibrators are 0.23 and 0.42 mg/L, respectively.

Discussion

Previous investigations have suggested that cystatin C might be a superior indicator of GFR compared with creatinine *[4, 5, 7]*. Early methods, including radial immunodiffusion and enzyme immunoassays, were generally slower, less precise, and too time consuming to perform in a routine biochemistry department. Therefore, the development of rapid, automated, and more-precise methods for determining cystatin C to evaluate the diagnostic potential of this analyte in the clinical situation has been important. Although there is one commercially available cystatin C method plus our own in-house assay, it is necessary to have as many methods as possible to make this assay both analytically and financially competitive for routine use. Here, we have evaluated the cystatin C method prepared by Behring Diagnostics based on a PENIA.

Table 3. Source of cystatin C calibrator material.

Method	Source of cystatin C	Assignment, mg/L	Recovered on BNA, %
Behring	Purified cystatin C from human urine	1.45	100
Dakopatts	Purified cystatin C from human material and assigned using recombinant	13.2	70
In-house	Recombinant cystatin C in horse serum matrix	10.0	50

This latex particle assay for cystatin C was sensitive, with good recovery and linearity, and had no major interferences. Our results show that this PENIA method agrees well with existing PETIA methods *[19, 20]*. Imprecision of 3–5% matches that of the in-house PETIA *[19]*, whereas Kyhse-Anderson et al. reported average imprecision of 2.0–3.2% *[20]*. The analyte had good stability in serum. We have shown that cystatin C is stable for 2 days at room temperature, 1 week at 4 °C, and 1 week at −20 °C. Although those measurements made after 2 months at −20 °C were statistically significant ($P < 0.05$), the actual change in values showed no trend with time and was not very great, i.e., 3.27 to 3.13 mg/L. The assay is precise and the between-assay variation at this concentration (4.2% CV) would suggest that this difference (2.5SD = 0.34 mg/L) will not be of clinical significance. Measuring cystatin C concentrations over 10 freeze/thaw cycles did not show any trends that were clinically or statistically significant. Kyhse-Anderson et al. *[20]* reported stability for only 5 days at 4 °C and three cycles of freeze/thaw, whereas Newman et al. *[19]* only reported on overnight stability at 4 °C and −20 °C.

Heparin and EDTA showed a statistically significant interference in cystatin C concentration in serum; the between-assay variation at this concentration (3.6%) would also suggest that this difference of 0.02 mg/L (2.5SD = 0.05 mg/L) will not be of clinical significance. However, although the cystatin C concentration in sodium citrate-anticoagulated plasma was 10% lower than serum-matched samples, these results can be accounted for by dilutional effects from the 1:10 ratio of anticoagulant to serum volume used in these Vacutainer Tubes.

The PENIA method shows less interference than those reported in the two PETIA methods *[19, 20]*: hemoglobin (≤1.0 g/L, ≤1.2 g/L), bilirubin (≤300 μmol/L, <150 μmol/L), triglycerides (≤10 mmol/L, 8.5 mmol/L), and rheumatoid factor (increased concentrations, ≤3230 kIU/L). Kyhse-Anderson et al. reported bilirubin interferences at 150–300 μmol/L, whereas we did not find this to be the case. We demonstrated that grossly hemolytic and lipemic samples do not interfere with this assay. Increased concentrations of rheumatoid factor showed no interference; neither did increased paraprotein concentrations.

Nephelometric assays have always been proposed as being potentially more sensitive than turbidimetric assays. Nephelometry monitors an increase in light intensity against a low background signal, and this gives nephelometric detection a theoretical edge. In practice, however, nonspecific background scatter in biological samples has required high sample predilutions, thus reducing the achievable detection limits to those of turbidimetric assays. Here we use a sample predilution that gives a lower sample fraction in the assay of 0.38% compared with the two turbidimetric assays (1.19% and 3.57%) with reduced interferences. There are other differences between the different methods, i.e., particles and antibody used; however, it is interesting to note that the PENIA appears to show less spectrophotometric interference and roughly equal imprecision and performance.

Comparison of this cystatin C assay with the two others, the in-house assay (mean difference = 1.73 ± 2.10) and the Dakopatts assay (mean difference = 1.13 ± 0.86), was good, with very few outliers (Fig. 3). Although there was a difference in slopes and intercepts, these could be accounted for when comparing the disagreement between the calibrator potencies and matrices of the three methods. On the Behring nephelometer system the recombinant protein calibrator was 50% of its assigned value, whereas the Dakopatts calibrator was 70%. Recalibration with the new assigned values allowed the slopes to become equal to 1.0. The disagreement in calibrator potencies must be due to the differences between recombinant and purified materials. Cystatin C does not have any glycosylation variance between materials; however, there may be unknown variations introduced during purification of cystatin C causing potency differences. Whatever the disagreement, a primary calibrator is required that can be an arbiter to assign secondary calibrators for measurement of cystatin C. This new marker requires an internationally agreed-upon reference preparation to allow direct comparison between methods and for future reporting of reference ranges. Pergande and Jung *[17]* reported a difference between male and female reference ranges (Table 1) with a urinary protein calibrator from Behringwerke; others did not find any significant sex differences *[15, 20]*. Our preliminary data are in agreement with Kyhse-Andersen et al. *[20]* and with the work of Löfberg and Grubb *[11]*, but further studies to establish a comprehensive reference range are necessary to explore whether there are no sex and age differences in cystatin C concentrations in a healthy population.

The nephelometer takes only ~10 s to dilute and transfer one sample to a cuvette, with a reading taken after 6 min. All samples in any one batch, up to a maximum of 75 samples, have to be diluted to 1:100 before the probe returns to the first diluted sample for transfer to a cuvette, obviously increasing the assay time for large batches. Having been mixed with the reagents,

the sample has a 6-min countdown before the reaction is completed. Each subsequent sample reading is available after 8 s. The sample volume used to make the first dilution is 80 μL. This is rather a large volume compared with 20 μL (Kyhse-Anderson et al. *[20]*) and 5 μL (Newman et al. *[19]*) and may be problematic for small-volume samples, i.e., patients in intensive therapy units and pediatrics.

The method comparison of 120 samples produced two outliers that could not be accounted for by analytical errors, and, unfortunately, because of a lack of patient information apart from creatinine values, no medical records could be searched for any clues to the poor correlation of either sample. However, the outliers showed up on only two of the three comparisons (Fig. 3), the common link being the Dakopatts method. Whereas both the in-house and Dakopatts assays share the same antibody, they do differ in the particles used, 80-nm chloromethyl styrene vs 38-nm carboxylate-modified particles. Whether the different conjugation procedures and particle surfaces contribute to such discrepancies is not known.

In conclusion, this new cystatin C assay was found to be a robust, fully automated, and rapid method, essential for the quick turnaround necessary in a routine hospital laboratory. Whereas other methods are adapted for routine turbidimetric analyzers, this method is specific for a nephelometric analyzer. A full age-related, sex-related reference range, with body mass indices, needs to be determined with this method and compared with others. Additionally, further prospective studies are required to monitor cystatin C concentration in patients with different renal pathologies, e.g., diabetic nephropathy to assess cystatin C as a potentially more sensitive and specific marker of GFR than creatinine.

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