Short Communication

Preliminary evaluation of the performance of a new, highly sensitive commercial immunoassay for serum ferritin determination

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Abstract

We evaluated the analytical performance of a new, commercial, fully automated immunoturbidimetric assay for the determination of ferritin [FER-Latex(X2)CN SEIKEN, Denka Seiken, Japan] in serum on the Olympus AU2700 analyzer. The new assay is a latex-enhanced turbidimetric immunoassay with an analysis time of 10 min. The linearity of the assay was confirmed up to 2505 pmol/L ($R^2 = 0.999$). The detection limit and the functional sensitivity were both 4.5 pmol/L. The intra- and inter-assay imprecision (CV) at 67, 506, 2186 pmol/L was $<1.8\%$ and $<2.5\%$, respectively. Verification of the traceability to a WHO standard (80/578) showed a recovery of 102.6% (target value 449 pmol/L). No hook effect was observed in samples containing up to 33,705 pmol/L. The assay showed good correlation with the Beckman Immage nephelometric system ($r = 0.999$). Hemoglobin (≤ 9.8 g/L), total bilirubin (≤ 113 μmol/L), conjugated bilirubin (≤ 109 μmol/L) and rheumatoid factor ($\leq 5.2 \times 10^5$ IU/L) did not interfere with the assay. The reference interval (2.5–97.5 percentile) was 72–521 pmol/L for men and 27–267 pmol/L for women. The reference interval in patients with anemia, malignant tumors and hemochromatosis was 5.6–52, 130–2436 and 1465–2903 pmol/L, respectively. On the basis of the receiver operating characteristic curve, the 90% sensitivity cut-off value to distinguish between patients with and without iron deficiency was 40 pmol/L. The new latex turbidimetric procedure for ferritin assay is an attractive alternative that avoids the need for dedicated instrumentation.

Keywords: automation; ferritin; turbidimetric immunoassay.

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Ferritin is a large, multifunctional, multisubunit protein that plays an important role in iron storage and metabolism. Its ability to sequester the element gives ferritin the double function of iron detoxification and reserve (1). Ferritin assays must have a broad dynamic range, as the serum concentrations can vary from >2247 pmol/L in some types of malignancies (2) to <23 pmol/L in iron-deficiency anemia (3). The determination of serum ferritin can be useful in detecting hemochromatosis in most subjects in a precirrhotic stage without irreversible complications (4). Finally, preliminary studies suggest that increased ferritin might be an independent predictor of premature coronary artery disease in male patients (5). Thus, precise measurement in the lower range is required for the diagnosis of iron-deficiency anemia, while a wide analytical range is useful for measurement in patients with tumors, inflammation, hemochromatosis or atherosclerosis.

The aim of this study was to evaluate a new serum ferritin assay, FER-Latex(X2)CN SEIKEN (Denka Seiken, Tokyo, Japan), on the Olympus AU 2700 analyzer (Olympus Diagnostics GmbH, Hamburg, Germany) by defining key performance characteristics, including detection limit, linearity on dilution, recovery, imprecision, comparison with a nephelometric method (Beckman Immage Assay, Beckman-Coulter, Fullerton, CA, USA), reference interval and cut-off value for iron-deficiency anemia. The method chosen for comparison is a well-established and widespread system for ferritin determination and the standards were calibrated against the 80/578 WHO Reference Preparation. The new assay is a latex-enhanced turbidimetric immunoassay that uses six different calibrators. Calibrator A is a 10 mmol/L HEPES buffer (pH 7.5). Calibrators B–F contain increasing concentrations of ferritin (67, 225, 450, 1124 and 2247 pmol/L) in HEPES buffer. These calibrators are traceable to the WHO standard (80/602).

The new FER-Latex method on the Olympus analyzer requires 15 μL of sample, 180 μL of Reagent 1 (170 mmol/L glycine buffer, pH 8.3) and 80 μL of Reagent 2. Reagent 2 consists of a suspension of polystyrene latex particles in 170 mmol/L glycine buffer (pH 7.3) coated with anti-human ferritin polyclonal antibodies. The latex particles are agglutinated according to the ferritin dose, which causes increased turbidity that is detected at 660 nm, with a total analysis time of 10 min. For comparison, the Beckman Immage assay was performed according to the manufacturer's recommendations.
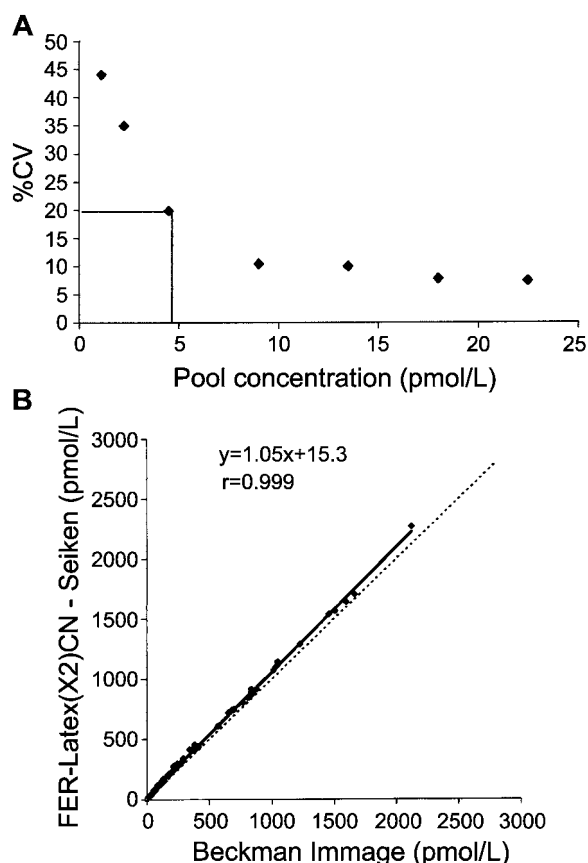
A

B

$y=1.05x+15.3$
$r=0.999$

Figure 1 Functional sensitivity (A) defined as the lowest measurable ferritin concentration with a CV of <20%. Comparison of the FER-Latex(X2)CN SEIKEN assay with the Beckman Immage assay (B) according to Passing-Bablok regression analysis. The dashed line represents the equivalence line.

We examined the linearity of the new method by analyzing 10 protein concentrations. Fresh plasma samples were used to prepare a high-concentration patient plasma pool (>2247 pmol/L). The undiluted pool and nine separate dilutions (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9) were assayed in duplicate in the same analytical run. Linear regression analysis was carried out and Pearson's correlation coefficient was calculated ($y=0.988x-5.512$; $R^2=0.999$). The results (percentage of theoretical concentrations) showed values between 96.2% and 98.6%, with a median bias of -2.8%; therefore, the method is linear within its measurement range (2505 pmol/L was the mean of the high-concentration pool). The standard error of regression ($S_{y/x}$) and t-test for the regression analysis were 3.082 and 343.4 ($p<0.001$), respectively. The minimum detectable ferritin concentration was assessed by 21 replicate measurements of the zero calibrator in a single run and defined as the ferritin value corresponding to a signal 2 SD greater than the mean found for this sample. On the basis of this procedure, the detection limit was 4.5 pmol/L for the assay. In addition, the functional sensitivity, defined as the concentration with an inter-assay imprecision (CV) <20%, was 4.5 pmol/L (Figure 1A). This coincidence of the functional sensitivity with the detection limit is probably a consequence of the reagents (buffers), which are unaffected by the matrix. This study

was performed by preparing seven serial dilutions of a ferritin pool, ranging from 1.1 to 23 pmol/L. To determine between-run imprecision, three pooled patient serum samples were aliquoted and frozen at $-20°C$. Two replicates/specimens were analyzed per run and one run per day for 21 days, using two reagent lots and two calibrations. CVs calculated from the means of each run were 2.5% (mean concentration 70 pmol/L), 1.5% (mean concentration 510 pmol/L) and 2.0% (mean concentration 2209 pmol/L). Within-run CVs were 1.8% (mean concentration 67 pmol/L), 0.5% (mean concentration 497 pmol/L) and 1.2% (mean concentration 2164 pmol/L). In this study, 21 replicates were analyzed in duplicate in the same analytical run.

The recovery of controls (Immuno-Q-sera II SEIKEN) calculated by the difference between expected and observed values (percentage recovery) was 98.1% (n=15; target value 243 pmol/L, mean±SD=238±4.3 pmol/L) and 99.3% (n=15; target value 944 pmol/L, mean±SD=937±9.7 pmol/L). Additional recovery experiments using two different concentrations of the calibrator in five samples gave a mean recovery from ten measurements of 98.8% (range 97.3–101.5%) of the calculated value. Verification of the traceability to the WHO standard, using the Beckman-Coulter Calibrator for ferritin (80/578 WHO standard), showed a recovery of 102.6% (n=15; target value 449 pmol/L, mean±SD=461±4.0 pmol/L). To determine whether highly increased concentrations of ferritin cause a ''hook effect'', a high-concentration serum pool was diluted with calibrator A. Final concentrations ranged from 2022 to 53,928 pmol/L and the samples were analyzed in duplicate. The results obtained from the undiluted samples were compared with those obtained from the same samples diluted to fall within the range of linearity. No hook effect was observed in samples containing up to 33,705 pmol/L of antigen, because the difference between the two determinations was below 5%. Passing and Bablok regression analysis (6) was used for method comparison between the FER-Latex(X2)CN SEIKEN assay and the Beckman Immage nephelometric system. To avoid any dilution, we limited the range for this experiment to the ranges reported for the two ferritin methods. For the comparison study, we used samples from 120 patients scheduled for ferritin determination for diagnostic purposes and/or monitoring of medical treatment. Passing and Bablok regression analysis (Figure 1B) revealed an intercept of 15.3 pmol/L (95% CI 13–20), a slope of 1.05 (95% CI 1.04–1.06), and a coefficient of correlation (r) of 0.999, suggesting that the values obtained with the new method are slightly higher than those obtained with the comparison assay. As a consequence the [(compared)–(comparison)] mean differences at the lower and higher reference limits for males and females (72, 27, 521 and 267 pmol/L) are 16, 17, 40 and 27 pmol/L, respectively.

Using a pool of samples with a mean concentration of 211 pmol/L of ferritin spiked with interferents and processed in duplicate did not affect results significantly, even though the concentrations of interferents

in spiked samples were 113 μmol/L total bilirubin (recovery 100.0%), 109 μmol/L conjugated bilirubin (recovery 101.0%), 9.8 g/L hemoglobin (recovery 103.0%) and 5.2×10^5 IU/L rheumatoid factor (recovery 102.8%). The spiking solutions were prepared from stock solutions containing known levels of interferents. Stored calibration curves were compared with fresh calibration curves. Two levels of controls and two human serum pools were analyzed at 1-week intervals. The calibration curve is stable for at least 1 month (error <5%).

Four groups of subjects entered the clinical evaluation study. Group 1 included 140 (70 males and 70 females; median age 39 years; range 21–53 years) healthy individuals using exclusion criteria as suggested in IFCC recommendations on the theory of reference values (7). The other three groups consisted of 80 iron-deficiency anemia patients, 80 subjects with a malignant tumor and 25 hemochromatosis patients. The three pathological groups were classified according to the following criteria: transferrin saturation <15% and hemoglobin <140 g/L in males or <120 g/L in females for iron-deficiency anemia patients; histolopathological findings for subjects with a malignant tumor; and homozygosity for the C282Y mutation of the *HFE* gene for patients with hemochromatosis. The procedures followed were in accordance with the current revision of the Helsinki Declaration (8). The reference interval for ferritin calculated by non-parametric determination of 2.5–97.5 percentiles was 72–521 pmol/L for men and 27–267 pmol/L for women. The reference interval in patients with anemia, malignant tumors and hemochromatosis was 5.6–52, 130–2436 and 1465–2903 pmol/L, respectively.

To determine the diagnostic accuracy of the assay for anemia, we performed receiver operating characteristic (ROC) plot analysis, and the area under the curve (AUC) was calculated. The cut-off value was determined at the 90% sensitivity criterion derived directly from the ROC curve. All probabilities were two-tailed, and p-values <0.05 were regarded as statistically significant. When distinguishing between patients with and without anemia (n=80 and n=100, respectively) the AUC (SE) was 0.967 (0.013) using the FER-Latex(X2)CN SEIKEN assay (95% CI, 0.929–0.988). The AUC (SE) was comparable using the Beckman Immage assay: 0.979 (0.015) (95% CI, 0.926–0.990). On the basis of the ROC curve, 40 pmol/L was the cut-off value for anaemia at 90% sensitivity (95% CI, 81.2–95.6) and 92% specificity (95% CI, 84.8–96.5) for the FER-Latex(X2)CN SEIKEN assay. Using the Beckman Immage assay, the cut-off value was 36 pmol/L at 90% sensitivity (95% CI, 82.4–96.1) and 91% specificity (95% CI, 83.9–94.5).

In conclusion, the method evaluated here can be operated on several common automated clinical chemistry analyzers; the new assay offers a high degree of reproducibility, together with an extended range, allowing direct measurement of the majority of the samples without further external sample dilution. No statistically significant bias in linearity was observed when samples were diluted. An analytical and functional sensitivity of 4.5 pmol/L and imprecision of CV<3% in all cases make the assay suitable for use in the diagnosis and monitoring of iron-deficiency anaemia. The wide analytical measurement range (4.5–2471 pmol/L) is useful for patients with tumors, inflammation or hemochromatosis. No substantial differences were observed by comparing results of the new turbidimetric assay with the Beckman nephelometric assay. In addition, the calibration curve shows good stability (1 month).

The new latex turbidimetric procedure for ferritin assay is an attractive alternative that avoids the need for dedicated instrumentation. Finally, this is an example of instrument consolidation without a deterioration in quality performance, an important goal for every clinical laboratory.

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