Latex Agglutination Test for Ferritin Measurement

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Ferritin is an indispensable parameter in the diagnosis of latent iron deficiency anemia or siderosis. In this study, we evaluated the utility of a reagent for ferritin measurement in a latex agglutination (LA) test, using general chemistry analyzers. The intraassay coefficient of variation (CV) was 0.8–3.4% and the interassay CV was 0.0–0.7%. Linearity was observed up to 1,100 ng/mL. The effective sensitivity value was 4.0 ng/mL. In addition, good results were obtained with the prozone test, the effects of interferences, correlation with the enzyme immunoassay (EIA) method, and functional assay sensitivity. A significant positive correlation with C-reactive protein ($r = 0.586$, $P < 0.001$) was found. When compared with liver-

related biochemical parameters (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) in patients with impaired liver function, ferritin varied in parallel with the liver-related parameters. This assay system was able to measure ferritin accurately over a wide range, and thus could be used to diagnose cancer, siderosis, and iron deficiency anemia. The LA assay system can be employed for measurement with general chemistry analyzers, with rapid and convenient execution. In addition, the LA test allows the simultaneous measurement of other markers of iron deficiency anemia, so clinicians can rapidly obtain examination results. *J. Clin. Lab. Anal.* 17:223–228, 2003. © 2003 Wiley-Liss, Inc.

Key words: ferritin; latex agglutination; general chemistry analyzer

INTRODUCTION

Ferritin is a soluble heme protein that is hollow inside to facilitate iron storage and channels. It has an approximate molecular weight of 440,000 and is composed of 24 subunits formed by heart (H) and liver (L) chains. Depending on the differences in the relative ratios of both chains, more than 20 tissue-specific isoforms with various isoelectric points are known (1–3). Large amounts of ferritin are found in the liver and spleen. It is also widely distributed in various other tissues, including the intestinal mucosa, placenta, heart, kidney, and red blood cells.

The ferritin level is related to the amount of deposited iron in the body (1 ng/mL of serum ferritin in adults = 8 mg of deposited iron) (4), and therefore ferritin measurement is indispensable in the diagnosis of latent iron deficiency anemia or siderosis. Moreover, ferritin measurement is also useful for monitoring therapeutic effects in acute hepatitis, acute and chronic myeloid leukemia, hepatoma, and pancreatic cancer (5–7). Ferritin measurement methods typically involve the use of special equipment.

In this study we evaluated the reagent for ferritin measurement by the latex agglutination (LA) test, with general chemistry analyzers.

MATERIALS AND METHODS

Measurement Principle

Ferritin was measured with an assay kit, using the LA test. When samples were mixed with latex particles to which anti-ferritin antibodies were adsorbed, an antigen-antibody reaction occurred between the ferritin in each sample and the anti-ferritin antibodies bonded to the latex particles. This reaction caused the agglutination reaction of latex particles, which was measured at a wavelength of 580 nm. Since the ferritin concentration in each sample was proportional to the change of absorbance, ferritin concentration was determined by constructing a calibration curve with known concentrations.

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Samples and Measurement Equipment

The samples consisted of sera from patients who were undergoing ferritin measurement at the Gunma University Hospital Clinical Laboratory Center, as well as sera obtained from staff during examinations. An interference check A plus was used to examine the effects of interference substances. Fer-Latex X2 Seiken® (Denka Seiken Co., Ltd.) was used as the measurement reagent. The E-test Tosoh II (ferritin)® immunoreaction reagent (Tosoh Co., Ltd.) was used in the conventional method. The measurement equipment consisted of a Hitachi 7170S type automatic analyzer (H-7170S) and an automatic EIA device, the AIA-1200i (Tosoh Co., Ltd.).

RESULTS

Precision

Twenty consecutive measurements were performed simultaneously using serum pools of three concentrations. The coefficient of variation (CV) was 0.7–3.4% when mean values of 15.1–441.2 ng/mL were measured. Moreover, the results of the intermittent measurements for 6 days showed CV values of 0.5–0.7% when mean values of 39.6–1009.5 ng/mL were measured (Table 1).

Linearity

Serial dilutions of concentrated serum pools were prepared with 0.01 M HEPES buffer containing 1%

bovine serum albumin (pH 7.5). Their concentrations were measured, and linearity was confirmed to be about 1,100 ng/mL (Fig. 1A).

Prozone

Serial dilutions of concentrated serum pools were prepared with 0.01 M HEPES buffer containing 1% bovine serum albumin (pH 7.5), and the concentrations were measured. Ferritin concentration plateaued at approximately 4,000 ng/mL. No prozone phenomenon was observed, even though the measurement was performed up to 20,000 ng/mL (Fig. 1B).

TABLE 1. Within-run and between-day precision (ng/mL)

Sample	Within-run precision			Between-day precision		
	A	B	C	A	B	C
n	20	20	20	6	6	6
Mean	16	104	411	40	790	1019
Min	14	103	404	40	784	1009
Max	16	105	415	40	796	1030
Range	2	2	11	0	12	21
SD	0.5	0.8	2.9	0.0	4.0	7.6
CV(%)	3.4	0.8	0.7	0.0	0.5	0.7

Within-run precision was evaluated by analyzing three levels of control in 20 replicates. Between-day precision was evaluated by analyzing three levels of control in triplicate for 6 days.

Fig. 1. **A:** Linearity. Dilution linearity was evaluated by linear regression analysis using high-range ferritin samples, and the respective diluted ferritin samples that covered the analytical range. The samples were prepared by diluting the high-ferritin pool with 0.01 M HEPES buffer containing 1% bovine serum albumin (pH 7.5). **B:** Prozone phenomenon. Measurement values of serial diluted samples became level at a ferritin concentration of approximately 4,000 ng/mL. The prozone phenomenon was not observed, even though measurement up to 20,000 ng/mL was performed.

Effects of Interferences

No effects were observed, even though the effects were examined to 18.6 mg/dL of free bilirubin, 22.4 mg/dL of conjugate bilirubin, 480 mg/dL of hemolyzed hemoglobin, and 1,670 mg/dL of chyle as formazin turbidity.

Lower Detection Limit

Serial dilutions of the standard solutions attached to the kit were prepared with 0.01 M HEPES buffer containing 1% bovine serum albumin (pH 7.5), and their concentrations were measured in 20 replicates. The lower detection limit was defined as the lowest ferritin concentration that could be distinguished from the zero calibrator (saline) with 99.9% certainty. The lower detection limit was determined to be 3.0 ng/mL (data not shown).

Effective Sensitivity

Serial dilutions of serum of known concentration were prepared with 0.01 M HEPES buffer containing 1% bovine serum albumin (pH 7.5), and their concentrations were measured for 20 days. When the precision profile was drawn with the mean measurement value and the CV, and the concentration of CV 20% was defined as the effective sensitivity, the results showed a value of 4.0 ng/mL (Fig. 2).

Correlation With Conventional Method

A total of 166 samples were analyzed, and a significant correlation was observed: $r = 0.9996$ ($P < 0.001$), and y (H-7170S) = $-9.9 + 1.1 \times (\text{AIA} - 1200i)$ (Fig. 3).

Distribution in Healthy Subjects

Sera were obtained from healthy subjects who had undergone an examination in the hospital, and whose measured values of liver functions, renal functions, and lipids were within the normal criteria for the hospital (hemoglobin concentration of ≥ 10.0 mg/mL by blood cell count). A difference between the sexes was found: 185.4 ± 123.0 ng/mL (mean $\pm$ SD) in males ($n = 141$ subjects, age range = 40 ± 10 years (mean $\pm$ SD)) and 38.0 ± 33.9 ng/mL in females ($n = 173$, age range = 37.2 ± 12.6 years). Moreover, after the abnormal data were excluded by the Grubbs Smirnov method, the reference criteria were calculated by the minimum skewness parametric method: 27–288 ng/mL for males, and 6–217 ng/mL for females (Fig. 4).

Fig. 2. Effective sensitivity. The functional assay sensitivity was assessed by analyzing serial dilutions of low-level control material in duplicate for 20 days. Functional sensitivity was 4 ng/mL, defined as the lowest measurable ferritin concentration with a CV of $< 20\%$.

Fig. 3. Comparison of methods. The Fer-Latex X2 Seiken[®] assay on the Hitachi 7170S analyzer was compared with the E-test Tosoh II (ferritin)[®] on the AIA-1200i system. The results were compared by linear regression.

Relationship With Diseases

A significant ($P < 0.001$) positive correlation was found between ferritin and the inflammation marker

Fig. 4. Distribution of ferritin levels in normal males and females. A difference between the sexes was observed: 185.4 ± 123.0 ng/mL in males ($n = 141$, mean age = 40 ± 10 years), and 38.0 ± 33.9 ng/mL in females ($n = 173$, mean age = 37.2 ± 12.6 years). After the abnormal data were excluded by the Grubbs Smirnov method, the reference criteria were calculated by the minimum skewness parametric method: 27–288 ng/mL for males, and 6–217 ng/mL for females.

C-reactive protein (CRP) ($n = 39$, $r = 0.586$; data not shown). Ferritin was compared with liver-related biochemical parameters (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) in patients with hepatocellular carcinoma, liver cirrhosis, drug-induced hepatitis, and pancreatic cancer, in the course of the diseases. It was found that ferritin varied in parallel with the liver-related parameters (Fig. 5).

DISCUSSION

Ferritin measurement typically requires the use of special equipment, along with RIA, EIA, and CLIA techniques. In this study, basic examinations of a reagent for ferritin measurement with LA were performed with the H-7170S general chemistry analyzer. Good results were obtained in terms of precision, linearity, effects of interference substances, the prozone test, and correlation with the conventional method.

Moreover, the measurement time was also reduced by about one-quarter (H-7170S: AIA 1200i), enabling clinicians to obtain test results rapidly. Furthermore, since the present technique avoids the problems of radioactive waste from RIA and waste (including reaction cuvettes) from EIA and CLIA, costs can be reduced.

With the current method, ferritin can be measured using general chemistry analyzers without any special equipment. In addition, since the ferritin measurement is performed with trace samples and other parameters, simultaneous measurements of serum iron and transfer-

rin as other markers of iron deficiency anemia are also possible.

The lower detection limit of 3.0 ng/mL and linearity to 1,100 ng/mL obtained with this kit are equal to those obtained with RIA or EIA (8,9). However, the ferritin standard varies depending on the measurement methods used (10–12). This is likely due to differences in standard ferritin composition among various measurement kits. While many standard ferritin is extracted from human liver, Hazard et al. (13) reported that iso-ferritin composition from the same organ varies depending on the extraction methods used. Therefore, we recommend that each institution define its own standard for ferritin measurement, whether the conventional method or the new method is used. Thus, it seemed necessary to define the standard in each institution even when the ferritin measurement is changed from the conventional method to this method.

Healthy female subjects had approximately one-fifth less ferritin than males, as a mean value. Normal distribution was observed in males, while left semilogarithmic distribution was found in females, suggesting that many healthy females have decreased or poorly deposited iron.

Serum ferritin is a useful marker for measuring amounts of deposited iron. Synthesis of apoferritin occurs in proportion to iron (14,15). Cytokines, including interleukin (IL)-1 and IL-6, promote the synthesis of apoferritin and CRP (16,17). In this study, a positive correlation between ferritin and the inflammation marker CRP was found. We also found that ferritin

Fig. 5. Comparison of the time course of ferritin levels with AST and ALT. The time course was compared with that of liver-related biochemical parameters (AST and ALT) in patients with hepatocellular carcinoma (A), liver cirrhosis (B), drug-induced hepatitis (C), and pancreatic cancer (D). Ferritin varied in parallel with related liver parameters.

varied in parallel with leaking enzymes from the liver of patients with impaired liver function. The results indicate that variations in serum ferritin concentrations during cell destruction can be used as a marker for a large variety of pathologic conditions, including various neoplastic diseases and systemic lupus erythematosus (18,19). It can also be used to assess the therapeutic effect of interferon treatment for hepatitis type C (20).

In conclusion, the LA test using general chemistry analyzers is a useful tool for measuring serum ferritin in patients with various disorders.

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