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Comparison of Manual and Automated ELISA Methods for Serum Ferritin Analysis

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Serum ferritin concentration is a sensitive measure of body iron stores. The aim of this study was to compare the performance of two commercially available enzyme-linked immunoassays (ELISAs) for serum ferritin: a widely used manual assay kit (Spectro Ferritin MT®), and a new fully automated assay (Immulite®). We analyzed serum samples from Moroccan school-aged children (n = 51) from a rural area with a high prevalence of iron deficiency anemia (IDA). Four replicates of each sample were analyzed using both assays. For the manual method, the interassay repeatability was 24%, 22%, and 11%, and intraassay precision was 18.3%, 9.2%, and 9.1% at increasing serum ferritin concentrations. Using the automated assay, the interassay repeatability was 7%, 6%, and 6%, and intraassay precision was 1.5%, 5.4%, and 5.5% at increasing serum ferritin concentrations. The two assays were well correlated ($y = 1.16x + 1.83$; $r = 0.98$). However, the limits of agreement (LOAs) were wide, particularly at low concentrations. A comparison of the assay results with recommended cutoffs for serum ferritin generated sharply different estimates of the prevalence of iron deficiency (ID) in the sample. We conclude that the automated assay has several potential advantages compared to the manual method, including better precision, less operator dependence, and faster sample throughput. J. Clin. Lab. Anal. 19:196–198, 2005. © 2005 Wiley-Liss, Inc.

Key words: immunoassay; ferritin; serum; automated; manual

INTRODUCTION

Iron deficiency anemia (IDA) is a major public health problem worldwide (1). Serum ferritin concentration is the recommended screening test to identify iron deficiency (ID), and a serum ferritin <15 μg/L in the presence of anemia indicates IDA (1,2). The utility of serum ferritin for identifying ID is well established, and its sensitivity and specificity may be as high as $\approx$92–98% compared to bone marrow biopsy (3). Several assays for serum ferritin are available, all of which are based on immunochemical principles. Few data are available comparing the performance of the available assays (4), particularly in children. In our previous large surveys of iron status in west and north Africa (5,6), we used a manual enzyme-linked immunoassay (ELISA) method to measure serum ferritin, but the assay is labor-intensive and highly operator-dependent, and its precision is variable at low concentrations. A fully automated, ELISA-based method has recently become available. In this study the performance of the automated assay was compared with a widely used manual serum ferritin assay.

MATERIALS AND METHODS

Sample Characterization

Whole blood was collected by venipuncture into EDTA-containing tubes in October 2003 from children in northern Morocco during a large cross-sectional screening of iron status. The mean age of the children was 9 years (range = 6–14 years). Blood samples were transported on ice to the regional hospital laboratory. After centrifugation on the day of collection, serum samples were aliquotted and frozen at –20°C until they were analyzed. The samples were defrosted and analyzed with the two serum ferritin methods on the same day.

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Laboratory Analyses

From the screening, we selected serum samples (n = 51) that represented a range of serum ferritin values of ≈0–100 μg/L. For the comparison study, serum ferritin was measured using two methods: a manual method (Spectro Ferritin MT®; Ramco Laboratories, Houston, TX), and a fully automated method (Immulite®; Diagnostics Products Corporation, Los Angeles, CA). Both assays were done following the manufacturer's instructions. For the Ramco assay, three-level WHO reference controls (WHO control serum; Ramco Laboratories, Houston, TX) were used (15 ± 6 μg/L, 83 ± 21 μg/L, and 330 ± 99 μg/L) as external controls. For the Immulite assay, three-level reference controls provided by the manufacturer were used (34 ± 3 μg/L, 135 ± 10 μg/L, and 287 ± 16 μg/L). The samples were analyzed in four replicates for each method, except for the controls, in which duplicates were analyzed.

Statistical Analyses

Data processing and statistics were done using Excel 2002 (Microsoft Inc. Redmond, WA) and SPSS 10.0 (SPSS Inc., Chicago, IL). Statistical analysis was done according to the method of Bland and Altman (7), with slight modifications. The absolute differences of the values in the samples analyzed by the two methods were calculated. The difference of the highest to the lowest value obtained with each method within the four replicates, as well as the difference of the center values of the four replicates was calculated. Limits of agreement (LOA) were calculated using

$$\delta - 2s = LOA_{low} \quad (1)$$

$$\delta + 2s = LOA_{high} \quad (2)$$

where δ is the mean of the difference between the two methods, and s is the standard deviation (SD) of this difference.

Interassay precision expressed as RSD of the mean of all performed measurements was calculated using the quality control sera for different levels. Intraassay precision expressed as RSD was based on four consecutive measurements of the same sample.

RESULTS

The interassay repeatability (n = 30) was 24%, 22%, and 11%, and the intraassay precision (n = 4) was 18.3%, 9.2%, and 9.1% for control sera at levels of 15, 83, and 330 μg/l using the manual assay. The interassay repeatability (n = 14) was 7%, 6%, and 6%, and the intraassay precision (n = 4) was 1.5%, 5.4%, and 5.5% for control sera at levels of 34, 135, and 287 μg/l using the automated assay. The slope (± SD), intercept (± SD), and correlation coefficient of the linear regression between the two methods was y = 1.16 (± 0.03)x + 1.83 (± 1.67) and r = 0.98 (manual = y, automated = x; individual data points calculated from the median of four replicates). Figure 1 shows the difference in the medians of the two methods (based on four replicates), including the LOAs (–8.5 ± 16.8). To investigate the stability of the replicate results, and hence the stability of the method, we calculated the difference of the extreme replicate values and the central values (x_1 and x_4 are the highest and lowest replicate values, respectively; Table 1). All reference control values were within the acceptable range, except for one high control measured by the manual assay.

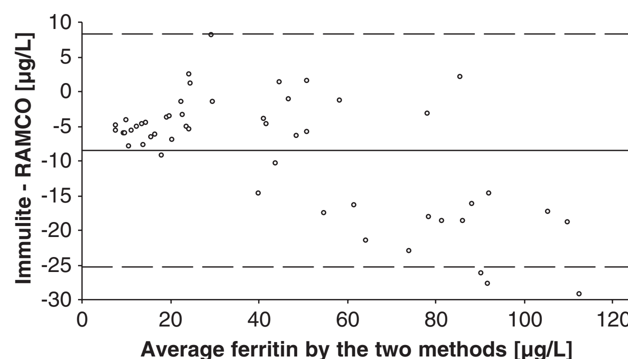

FIG. 1. Difference in the medians of the manual and automated assays for serum ferritin concentration (based on four replicates). The limits of agreement are indicated by the dashed lines (— — —).

TABLE 1. The mean and SD of the differences between the highest and the lowest replicate values and the two center values for four replicates of serum ferritin concentration, measured using the manual and automated assays

	Ramco (μg/L)	Immulite (μg/L)
$x_2 – x_3$[a]	3.96	0.95
$x_1 – x_4$[a]	11.62	3.10
SD[a]	5.33	1.39
$x_2 – x_3$[b]	1.43	0.26
$x_1 – x_4$[b]	7.61	1.21
SD[b]	3.49	0.54

[a]Mean calculated for all samples (n = 51).
[b]Mean calculated for samples with ferritin concentration = 20 μg/L (n = 16).

DISCUSSION

The LOAs between the two assays were wide, particularly considering that the recommended WHO

cutoff value for serum ferritin for identifying ID is 15 µg/L (1). In screening for ID in our sample using this cutoff, the two assays produced sharply different estimates of prevalence: the prevalence of ID was 13.7% using the manual assay, and 27.5% using the automated assay. With the use of these assays, measurements of longitudinal changes in iron status within a population group are minimally affected by bias.

Our findings suggest that the automated ELISA method for serum ferritin has several advantages over the manual assay, including 1) better precision, which is particularly important at low serum ferritin concentrations; 2) less operator dependence; and 3) faster sample throughput.

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