Interpreting Results of Coulometry and Immunoprecipitation in Diagnosing Iron Disorders


Concentration of iron in plasma, total iron-binding capacity (TIBC), and transferrin saturation are often determined by standard spectrophotometric methods, but iron concentration may be quantified by immunoprecipitation or, electrochemically, by controlled-potential coulometry. Because these iron assays do not all measure the same form(s) of iron, we studied subjects in various states of iron nutrition: normal adults, iron-deficient patients, thalassemia patients with unsaturated transferrin or oversaturated transferrin, and patients with idiopathic hemochromatosis. The spectrophotometric and coulometric methods detected essentially all non-heme iron in plasma; results correlated well but showed a negative bias toward the coulometric method. Results by an immunoprecipitation procedure, which measures only transferrin-bound iron, correlated well with those obtained coulometrically but were slightly higher than the latter. The characteristics of the various methods for iron must be understood by the clinical laboratory if diagnosis of iron disorders is to be accurate.

Iron in serum or plasma is most often assayed by deproteinizing the sample to release ferric iron, reducing this to ferrous iron, forming an iron chelate, and measuring this chromophore spectrophotometrically (1). Total iron-binding capacity (TIBC) is usually determined by adding to the serum or plasma excess ferric iron to saturate the transferrin, then removing the excess iron with magnesium carbonate, and assaying the total transferrin-bound iron (2). Alternatively, iron concentration may be assayed directly in serum or plasma by controlled-potential coulometry. In this system, TIBC can be determined directly by selectively saturating the transferrin with an iron-loaded resin and re-assaying the treated specimen. Recently, a method for determining iron by immunoprecipitation has been suggested which measures only transferrin-bound iron (3).

For this investigation, we compared results of a coulometric assay for serum iron (4) and those of an immunoprecipitation assay, both conducted on subjects or patients in various states of iron nutrition. Our objective was not so much to compare one assay method with another as to evaluate the results obtained by controlled-potential coulometry for the diagnosis of iron disorders.

Materials and Methods

For the iron assay, we used a Ferrochem™ Model 3050 Serum Iron/TIBC Analyzer (Environmental Sciences Associates, Bedford, MA 01730) (4), which measures iron electrochemically by controlled-potential coulometry. We mixed the specimen with the manufacturer's proprietary "Ironex" reagent, containing hydrogen chloride and n-propanol to solubilize the protein and release all transferrin-bound iron; most other non-heme iron is also released. In the TIBC assay, the commercially prepared cups contain an iron-loaded resin used to saturate specifically the transferrin in the specimen; the resin is then removed by centrifugation, leaving transferrin fully saturated with iron. Both the treated and the untreated serum specimen are then assayed for iron content. We also assayed iron and TIBC by an immunoprecipitation procedure described elsewhere (3).

We assayed specimens from five different groups of subjects: a control group of 15 healthy adults, 12 iron-deficient patients, six thalassemia patients with unsaturated transferrin, 10 thalassemia patients with over-saturated transferrin, and eight subjects with idiopathic hemochromatosis.

Results and Discussion

The Ferrochem Iron Analyzer detects essentially all non-heme iron in normal plasma, and results obtained with it should agree well with those obtained by the baphenanthrolone spectrophotometric procedure described by the International Committee for Standardization in Hematology (1,2). In fact, results for aqueous ferric chloride standards [100, 200, 300 μg/dL (17.9, 35.8, 53.7 μmol/L)] agreed well between the two assays (r = 0.998, y = 0.950x + 5.00), and the use of these assays to quantify plasma iron in 15 normal, healthy adults gave a correlation coefficient (r) of 0.965 (y = 0.876x – 9.40). On the other hand, the mean determined with the Ferrochem assay, 77.3 μg/dL (13.9 mmol/L), was 22% less than the baphenanthrolone mean of 98.9 μg/dL (17.8 μmol/L), a distinctly negative bias. Because the two methods are intended to measure the same non-heme iron, results from all of the subjects in the several groups ought to correlate well; indeed, results were comparable (n = 51, r = 0.966, y = 0.910x – 1.15) with a negative bias similar to that seen for only the healthy subjects, x = 157 vs 115 μg/dL (22.9 vs 20.7 μmol/L), for coulometry vs spectrophotometry. The relatively few subjects available in most of the study groups precluded calculation of significant regressions for each individual group.

Table 1 summarizes linearity and precision of the two methods. To verify that hemoglobin iron was not included in the iron measured by the coulometric or immunoprecipitation assays, we repeatedly froze and thawed hemolyzed plasma that contained 50 to 500 μg of hemoglobin Fe per deciliter (9-90 mmol/L) before analysis. Neither the coulometric nor the immunoprecipitation method detected the heme-iron present in hemolyzed preparations.

The coulometric and immunoprecipitation assays would be expected to provide quantitatively different results because the immunoprecipitation procedure measures specifi-

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**Table 1. Linearity and Within-Run Precision of Coulometric and Immunoprecipitation Methods for Measuring Iron and TIBC in Plasma**

<table>
<thead>
<tr>
<th>Method</th>
<th>CV, %</th>
<th>Linearity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coulometric Iron*</td>
<td>3.42 (x̄ = 67.2)</td>
<td>0–1000</td>
</tr>
<tr>
<td>TIBC*</td>
<td>1.43 (x̄ = 363.2)</td>
<td></td>
</tr>
<tr>
<td>Immunoprecipitation Iron*</td>
<td>11.6 (x̄ = 74.9)</td>
<td>0–800</td>
</tr>
<tr>
<td>TIBC*</td>
<td>6.18 (x̄ = 304.5)</td>
<td></td>
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</tbody>
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*μg/dL

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cally transferrin-bound iron, whereas the coulometric procedure detects non-heme iron bound to transferrin and other proteins, e.g., ferritin iron and albumin-bound iron (if the latter exists). A plot of the Ferrochem and the immunoprecipitation results for iron in plasma (Figure 1, left) illustrates a slightly negative bias between the Ferrochem results and the immunoprecipitation results, except for samples from the iron-deficient subjects. Because the hemochromatosis patients in whom the transferrin was essentially saturated showed a similar slight negative bias, we conclude that the bias occurs throughout the assay range. On the other hand, the high coulometric results for two thalassemia patients with iron overload suggest that the concentration of iron in plasma exceeded the binding capacity of transferrin and would have been detected electrochemically but not by immunoprecipitation. One might have expected these same high values for patients with hemochromatosis, but they apparently did not have iron overload.

Determinations of TIBC from the normal and iron-deficient subjects by the two methods obviously agreed well between the two methods (Figure 1, middle). Results from many of the thalassemia and hemochromatosis patients, as expected, showed a positive bias with the Ferrochem method. We attribute this to the presence and detection of non-transferrin-bound iron, which was not measurable by immunoprecipitation. In such cases, the less-specific Ferrochem method may be more useful in diagnosing iron overload because it detects total non-heme iron.

The percentage transferrin saturation in the 15 normal subjects correlated well between the Ferrochem and immunoprecipitation procedures (Figure 1, left; \( r = 0.948, y = 0.980x - 14.56 \)), but the former again showed a negative bias (22.7% vs 28.2%, respectively). Given that the percentage of transferrin saturation is a ratio of iron concentration to total iron-binding capacity, it is not surprising that the thalassemia and hemochromatosis patients showed a negative bias by the Ferrochem method, owing to a falsely high TIBC combined with a low bias for the plasma iron.

Particular characteristics regarding analyte specificity of different iron methods should be appreciated by the clinical laboratory, and results may not always be straightforward. In a recent case seen in this laboratory, the Ferrochem method gave a result for transferrin saturation >100%. Presumably, the transferrin in the specimen was oversaturated such that excess iron was adsorbed to rather than released from the iron-containing resin used in the TIBC assay. By contrast, the immunoprecipitation method may be of clinical value owing to its capacity to measure transferrin-bound iron specifically. This method may be of value in clinical diagnosis of iron overload due to hemochromatosis where, with the spectrophotometric method, for example, precipitation with magnesium carbonate causes removal of more than ionic iron, giving a falsely low result.

Clearly, not all iron assays measure the same form(s) of iron. Therefore, the accurate laboratory diagnosis of an iron disorder may require knowledge of exactly which form(s) of iron are detected by any given method. Moreover, multiple iron tests may be required to characterize fully some iron disorders, given the complexity of iron metabolism (5).

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References