Given that the serum kinetics of myoglobin and CA III are quite equivalent \cite{18, 19}, the ratio of serum myoglobin and CA III originating from skeletal muscle stays fairly constant after skeletal muscle damage \cite{5, 20}, but increases after injury to myocardium \cite{5}. We conclude from our studies that the myoglobin/CA III ratio is more sensitive than CK or CK-MB in the detection of early AMI, gives a clear advantage over the measurement of myoglobin alone, and thus provides a tool for early triage of patients. According to published methodology \cite{8}, simultaneous measurements of myoglobin and CA III from the same sample appear to require about the same amount of laboratory work as for each analyte individually. A limiting factor in the wide use of the method \cite{8} is the time required for the assay (3 h). Development of a rapid assay method for myoglobin/CA III ratio is in progress.

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References


Recently, we published a report showing poor performance of routine iron methods used in clinical laboratories \cite{1}. Compared with values obtained with the Reference Method of the International Committee for Standardization in Haematology (ICSH) \cite{2}, results obtained by these methods were generally lower throughout the entire measurement range. However, the poorest correlations were observed for iron values <750 µg/L. We listed possible causes for these discrepancies, with particular emphasis on incomplete dissociation of iron from its carrier proteins.

A moderately acidic pH is required to dissociate iron from transferrin. The pH of this step, however, varies among methods; some use a pH as high as 5.0. In the study reported here, we determined iron in patients’ serum samples with the Reference Method, an automated iron method distributed by Synermed (Montreal, Canada), and the manual method of Ceriotti and Ceriotti \cite{3}.

All patients’ specimens used for iron determinations were serum samples prepared from whole blood collected without anticoagulants. Specimens were collected from both hospitalized and ambulatory patients, for whom other serum laboratory tests had been ordered. The serum specimens were stored frozen at −20 °C until analysis. All procedures were performed in accordance with the ethical standards of our institution.

The Reference Method we used was the updated ICSH method \cite{2}, which uses hydrochloric and trichloroacetic acids for protein precipitation, thioglycolic acid as a reducing agent, and ferrozine as chromogen. The method was standardized with iron standards prepared from Standard Reference Material (SRM) 937 [obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, MD)] dissolved in HCl, 5 mmol/L. The absorbance of the iron–ferrozine complex was measured with a Lambda 5 ultraviolet/visible spectrophotometer (Perkin-Elmer, Norwalk, CT). A reagent blank was included in each run to check reagents for iron contamination. The ICSH method is subject to a 2.5% volume displacement error caused by the precipitation of proteins. We corrected all values for this error.

The Synermed Iron-600 method was performed as described in the manufacturer’s instructions with a BM/Hitachi 717 analyzer (Boehringer Mannheim, Indianapolis, IN). The pH of Reagent 1 (buffer) was 2.8 and of Reagent 2 (Ferene S/ascorbic
acid), 2.5; the final pH of the reaction mixture including serum was 2.8–2.9.

The manual method by Ceriotti and Ceriotti [3] was performed as published. The final reaction mixture containing serum had a pH of 1.6–1.9 (depending on the serum specimen). At this pH, the serum iron is released from transferrin and reduced with a solution of HCl and ascorbic acid. The free iron is then complexed with ferrozine to give a colored product, which is measured spectrophotometrically at 562 nm. As a separate experiment, we modified this method by adding glycine, 0.1 mol/L, to the HCl/ascorbic acid reagent to buffer the final reaction mixture pH (including serum) at 2.0 ± 0.1. All other steps in the modified method were carried out as in the original version.

All glassware used for preparation of iron reagents and standards was soaked at least 6 h in HCl, 2 mol/L, and then rinsed five times with deionized water before use.

Ferritin values were determined with the DPC Immulite instrument/reagent system (Diagnostic Products Corp., Los Angeles, CA). This method is a solid-phase, two-site chemiluminescence enzyme immunoassay designed for the quantitative measurement of ferritin in serum.

In our previous communication [1], we reported a marked negative bias for the Synermed method compared with the ICSH method. Since then, the manufacturer has modified the method used to adjust the reagent pH. The modified method showed good correlation with the ICSH method for specimens with ferritin values <1200 μg/L (Fig. 1A). For specimens with ferritin values >1200 μg/L, the ICSH method showed a positive bias for most values (Fig. 1B).

Ceriotti and Ceriotti [3] recommended an iron method without protein precipitation and with a final reaction pH of 1.6–1.9, depending on the serum sample. For specimens with ferritin <1200 μg/L, iron values by this method were consistently lower than the values obtained with the ICSH method (Fig. 1C), and were not as close to the ICSH values as those obtained with the new Synermed method. For our population of specimens, this negative bias was apparent throughout the measurement range up to 2000 μg/L. These lower results may possibly be attributable to the pH of the reaction mixture, which

![Fig. 1. Comparison of 1990 ICSH Reference Method and Synermed method (A) for specimens with ferritin <1200 μg/L and (B) for specimens with ferritin >1200 μg/L, and comparison of 1990 ICSH Reference Method and (C) unmodified Ceriotti method for specimens with ferritin <1200 μg/L and (D) modified Ceriotti method as described in text for specimens with ferritin values <1200 μg/L.](image-url)
was as low as 1.6 after addition of serum specimens. The ferrozine–iron complex does not develop full color at pH ≤1.65 [3] and, because the Ceriotti reagent is not buffered, small pH changes caused by different buffer capacities of different serum specimens may cause variability in pH and therefore in test results.

We therefore modified the iron reagent by adding 0.1 mol/L glycine to the HCl/ascorbic acid reagent; this addition provides a buffered pH of ~2.0 for the final reaction mixture with serum. Comparison of values obtained with this method modification gave results much closer to those obtained with the ICSH method (Fig. 1D).

Another possible cause for discrepancies in iron values is interference by ferritin, especially in serum samples with high concentrations of this iron-storage protein. Therefore, we also compared iron values between methods for specimens grouped with ferritin concentrations <1200 µg/L and >1200 µg/L. Fig. 1 (A and D) suggests that no ferritin interference occurs with the Synermed, Ceriotti, and ICSH methods in serum with ferritin concentrations <1200 µg/L. In the presence of higher ferritin concentrations, interference appears to be present only with the ICSH method. Unfortunately, this statement cannot be experimentally confirmed because we were unable to obtain a human serum ferritin preparation sufficiently concentrated to add to normal serum specimens. The interference observed with the ICSH method most probably results from the release of iron from serum ferritin because of the lower pH value of the reaction mixture and the harsh treatment of protein denaturation by precipitation with HCl and trichloroacetic acid.

Increase of serum iron values by nontransferrin iron was also reported by Huebers et al. [4]. Yamanishi also found interference by ferritin in the 1978 ICSH method (unpublished; submitted for publication). In the latter study, interference by ferritin was demonstrated by measuring serum iron before and after absorption of ferritin with solid-phase anti-ferritin antibodies.

The adjustment of pH in the iron reagents is critical for good recovery of iron in serum. Appropriate modifications of the reaction pH improved the accuracy of the Synermed and Ceriotti methods to a degree that is acceptable for use in the clinical laboratory. The ICSH method is subject to interference by ferritin when present in concentrations >1200 µg/L. This observation casts doubt on the suitability of the ICSH method as a Reference Method for the measurement of serum iron in the presence of high ferritin concentrations. Furthermore, the differences in results obtained with the two versions of the ICSH method [2, 5] that we reported previously [1] require clarification. Thus, we renew our call [1, 6] for a new Reference Method for measuring serum iron that meets today’s need for accuracy and precision.

References