Adaptation of the Zak-Epstein Automated Micromethod for Serum Iron to Determine Iron-Binding Capacity and Urinary Iron

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An automated method has been developed for determining serum iron-binding capacity and urinary iron as well as serum iron. After preliminary preparation, the sample is treated with ascorbic acid in hydrochloric acid to release and reduce protein-bound iron, then singly dialyzed into an acetate buffer and reacted with 4,7-diphenyl-1,10-phenanthroline (bathophenanthroline) to form a pink color proportionate to the amount of iron present. The sample passes through a 15-mm flow cuvet and is read at 537.5 nm, with the range expander set at either 2X or 4X.

A rapid, uncomplicated, and reproducible method for determining iron in body fluids has been needed for some time as a research tool and as an aid in diagnosis of disease involving the iron status of the body. Trinder (1) developed the use of sulfonated bathophenanthroline, which was extensively studied by Blair and Diehl (2), in iron determination. Zak and Epstein (3) incorporated these findings into an automated procedure for determining serum iron. This has now been modified to accommodate a semiautomated determination of serum iron-binding capacity and urinary iron.

Experimental

Reagents

(a) Ascorbic acid (1 g/100 ml) in 1N HCl.
(b) Acetate buffer, pH 4.65, 1M. Mix 500 ml of 1M sodium acetate (82.1 g/liter) and 500 ml of 1M acetic acid (57 ml/liter).
(c) 4,7-Diphenyl-1,10-phenanthroline sulfonate solution, 20 mg/100 ml of deionized water.
(d) Magnesium carbonate, light.
(e) Hydrochloric acid, 1.0N.
(f) Stock iron standards, 0.1 mg of iron per ml.
(g) Iron working standards, 0–500 μg/100 ml, prepared in 10-μg increments up to 50 μg/100 ml and 100-μg increments from 100 to 500 μg/100 ml.

Procedure

Serum iron and iron-binding capacity. One milliliter of clear nonhemolyzed serum for serum iron determination (3) is placed in an AutoAnalyzer cup, and 1 ml of serum is added to a centrifuge tube containing 2 ml of the standard (500 μg iron/100 ml). The latter is used to determine iron-binding capacity. The centrifuge tube containing serum with added iron is thoroughly mixed and allowed to stand at room temperature for 5 min, then 0.2 g of light magnesium carbonate is added. The mixture is agitated occasionally during the following 30 min and then centrifuged at 1500 rpm for 5 min or until clear. One milliliter of the clear supernatant fluid is transferred to an AutoAnalyzer cup for determining total serum iron-binding capacity. From here on the procedure is the same as for serum iron except that the result must be multiplied by three to correct for dilution (4).

Urinary iron. Two milliliters of urine is added to a centrifuge tube containing 2 ml of 1.0N HCl, and this mixture is agitated to drive off excess CO₂. After complete removal of CO₂, the acidified urine is centrifuged at 1500 rpm for 5 min or until clear. One milliliter of the clear, acidified urine is transferred to an AutoAnalyzer cup. The procedure from here on is exactly like that for serum iron and total iron-binding capacity except that the results must be multiplied by two to correct for dilution and the range expander must be set at 4X when urine samples are being analyzed.

For the best accuracy, urine blanks should be

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run to correct errors caused by differences in concentration and urinary pigmentation.

**Results and Discussion**

For the most accurate determination of serum iron, iron-binding capacity, or urinary iron with this procedure, the samples should be run at a rate of 30 per hour with a sample-to-wash ratio of 1:2.

Two delay coils (Fig. 1) of approximately 6 ml capacity are used in the system to allow 10 min for the release and reduction of iron in the sample by reaction with hydrochloric and ascorbic acids. The entire procedure takes 18 min.

By changing the tubing size and by single dialysis, the Zak-Epstein method was further modified so that smaller samples could be used.

Precision and accuracy of the procedure were determined for three groups of urine samples containing 10, 18, and 27 μg of iron per 100 ml. Each sample was analyzed 10 times to determine the precision of the procedure. Samples were in a random sequence, to avoid error caused by carry-over and variations in the AutoAnalyzer. The means and standard deviations were 10.2 ± 1.1, 17.7 ± 1.3, and 27.1 ± 0.8 μg/100 ml for the 10, 18, and 27 μg/100 ml samples, respectively.

An attempt was made to check the results obtained with this technique with those obtained by atomic absorption. However, at these low concentrations, atomic absorption techniques often did not detect iron, and when iron was detected, the results were not reproducible.

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**References**


