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Rapid methods for the measurement of serum iron can aid clinicians in evaluating the iron status of patients and for confirming cases of accidental iron poisoning. The proposed method, using a surfactant, under reducing and acidic conditions is rapid and sensitive. The proposed procedure compares favorably with the reference method, r=0.995 (n=28). High levels of bilirubin and hemoglobin do not interfere with the proposed technique. The interference by copper has been masked by using thiourea in the procedure. This technique is easily adaptable for use in clinical laboratories or in the field where instrumentation permits.

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Short Communication

Rapid spectrophotometric assay of serum iron *

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Introduction

Many manual methods for the measurements of serum iron are labor-intensive [1,2]. Methods that utilize automated instrumentation often are not cost effective for a small laboratory or clinic setting and require highly specialized training [3,4]. We have developed a simple, rapid and highly accurate method for measuring serum iron that provides results within a few minutes using the surfactant, polyoxyethylene (20) sorbitan monolaurate (Tween-20). These measurements were made under acidic and reducing conditions.

Materials and methods

Materials

Most of the chemicals used were purchased as iron-free reagents (G. Frederick Smith Co., Columbus, OH). Water used in reagent preparation was deionized and further purified to 18 MΩ resistance using a four-cartridge purification system (Millipore Corp., Bedford, MA). The iron-releasing reagent was made to contain 0.5% Tween-20 (v/v), 0.5% thiourea (w/v) and 0.1% ascorbic acid (w/v), all dissolved in 0.1 mol/l HCl. This reagent was stable 4–5 days if kept refrigerated. The color-development reagent contained 0.25 g/l bathophenanthrolene sulfonate, disodium salt dissolved in 2.0 mol/l sodium acetate. Standard solutions of iron and copper contained 2.0 g/l of each element, respectively. Spectrophotometry was performed on the Beckman DU-7 spectrophotometer (Beckman Instruments, Fullerton, CA). Horse serum standardized by the reference method was used as a source of iron binding proteins [5]. Hemoglobin was prepared and standardized according

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to the method of Crosby [6]. Standard amounts of bilirubin were solubilized in dimethyl sulfoxide.

Method

The following were added to appropriately labeled small plastic test tubes: 0.1 ml serum, standard or water, 0.7 ml iron-releasing reagent and 0.2 ml color-development reagent. A serum blank was prepared by adding to another test tube, 0.1 ml serum, 0.7 ml iron-releasing reagent and 0.2 ml of 2.0 mol/l sodium acetate. The contents were mixed and allowed to stand at least 5 min, after which, they were transferred to matched absorption cells and the absorbance was measured against water with the spectrophotometer calibrated at 535 nm. Corrected absorbance readings were obtained by subtracting the serum blank and reagent blank readings from that of the serum unknown and the reagent blank from that of the standard. From these corrected readings, the values of the iron content in the specimens were calculated.

Data analysis

Data analysis was facilitated using a VAX computer employing data reduction procedures described by Ryan et al. [7].

Results

The rate-limiting step in the procedure, the release of protein bound iron, was easily accomplished by using the surfactant, Tween-20, in an acidic medium under reducing conditions. An evaluation of the time required for iron to complex with bathophenanthroline sulfonate was made. We observed that the color formed by the iron-chromogen complex was instantaneous and was stable for at least 4 h.

The analysis for iron using the proposed technique was compared with that of the reference method [5]. We analyzed 28 specimens for iron content and found mean values of 1.31 and 1.27 mg/l for the reference and proposed techniques, respectively. The agreement between the two methods was excellent, $r = 0.985$. The slope of the regression line was 0.975 with a y-intercept of 0.08 mg/l.

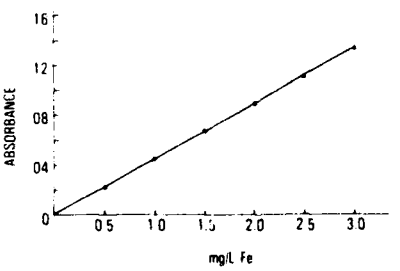

Fig. 1. Recovery of iron from spiked serum.

TABLE I

Evaluation of the adequacy of the concentration of the iron-releasing reagent

ml serum/test	Fe recovered (% control ± SD)
0.1	100.0 ± 0.0
0.2	99.3 ± 0.2
0.3	101.1 ± 2.3
0.4	97.1 ± 1.9
0.5	102.4 ± 3.1
0.6	99.9 ± 0.5
0.7	103.7 ± 1.6
0.8	93.6 ± 1.9
1.0	76.3 ± 3.3

The linearity of recovery was determined by adding known quantities of iron to a constant volume of serum and then measuring iron in the specimens using the proposed technique. Fig. 1 shows a recovery curve of serum spiked with iron. Recovery of iron was linear for values of iron between 0.5 and 3.0 mg/l.

The adequacy of the concentration of iron releasing and complexing reagents was determined by adding incremental amounts serum to the reaction mixture. The amounts of all other reagents were kept constant and the total volume of the reaction mixture adjusted to a final volume of 2 ml with water. Measurement for iron was made as described above. We found that the amount of iron recovered was essentially the same as that predicted for volumes up to 0.7 ml serum. These findings are summarized in Table I.

Hemoglobin (Hb), the major iron-containing protein in blood, and bilirubin, a plasma pigment that is elevated in certain disease states, were evaluated for

TABLE II

Serum iron values with time of measurement

	mg Fe/l				
	Day 0	Day 1	Day 2	Day 3	Day 6
	0.70	0.69	0.66	0.71	0.68
	0.70	0.66	0.67	0.71	0.68
	0.70	0.70	0.67	0.71	0.69
	0.73	0.66	0.69	0.71	0.69
	0.73	0.71	0.73	0.71	0.71
	0.73	0.69	0.71	0.71	0.72
	0.70	0.66	0.75	0.71	0.71
	0.73	0.70	0.73	0.71	0.70
	0.74	0.71	0.71	0.70	0.71
	0.73	0.59	0.67	0.81	0.69
$\bar{X}$	0.72	0.69	0.70	0.71	0.70
SD	0.02	0.02	0.03	0.03	0.01

TABLE III

The masking effect of thiourea on copper

mg/l added copper	% Control ± SD	
	without thiourea	with thiourea
1.0	163 ± 6.4	99.9 ± 1.3
2.0	184 ± 9.6	99.3 ± 1.3
3.0	230 ± 3.3	95.6 ± 1.5
4.0	265 ± 16.5	92.9 ± 1.1

interference with the proposed procedure. Incremental amounts of Hb prepared according to the method of Crosby [6] were added to serum and the test for iron was performed. There was no significant release of Hb bound iron up to 5.0 g/l Hb when added to control serum containing 1.55 mg/l iron. In triplicate analyses, the mean recovery of iron was 1.55 mg/l ± 0.05 (SD) ($n = 7$). Known amounts of bilirubin dissolved in dimethyl sulfoxide were added to control serum containing 2.01 mg/l iron which was then tested in triplicate for iron content. No significant interference was found due to bilirubin in amounts up to 20 mg/l. The mean recovery of iron was 2.04 mg/l ± 0.03 (SD) ($n = 7$).

Changes in serum iron values with respect to time were made on a test specimen. Replicate analyses for iron were performed on a pooled serum specimen over a 6 day period using the proposed technique. We found no significant differences in iron values for the within-day and day-to-day determinations. These data are summarized in Table II.

We evaluated the influence of copper on the measurement of iron under these reaction conditions by adding known amounts of copper. Bakker et al. found that copper interfered with the measurement of iron when using a ferrozine-based assay. They negated the influence of copper by making the reaction mixture 0.66 mmol/l with respect to thiourea [8]. In the proposed method we added 5.0 g/l thiourea to the iron-releasing reagent and the influence of copper was effectively masked for values up to 4 mg/l. These data, provided in Table III, show the influence of copper on the measurement of iron in the absence and presence of thiourea.

Discussion

Uncomplicated and reliable methods for measuring analytes often find acceptance in the clinical laboratory. The method presented here should fall into this category. In summary, this technique for estimating serum iron levels is easily adaptable for use in clinical laboratories or in the field where instrumentation permits. Results can be obtained almost immediately enabling the physician to more quickly assess the iron status of the patient and begin the most appropriate treatment.

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