yields a clear, protein-free supernate, the proteins being completely precipitated as a coherent, gelatinous mass. Transfer 0.8 mL of the supernate to another tube. Add 0.2 mL of phosphate buffer, pH 12.0 (per liter, 0.5 mol of Na2HPO4 and 0.5 mol of NaOH) and incubate at 37 °C.

The resulting mixture is effectively buffered at pH 11.5 with a difference not exceeding ±0.02 pH units, whether aqueous creatinine standard or serum or urine specimens are being used. After 25 min measure the absorbance of the sample at 525 nm vs water in a 10-mm cuvet. At 500 nm, the maximum-absorption wavelength, absorbance from creatinine-free serum specimens is significantly less than that obtained with water, a difference that progressively disappears at higher wavelengths until finally it is zero in all instances at 525 nm. The resulting color is stable for at least an hour and is linearly related to concentration from 0.050 to 1.500 A for aqueous creatinine standards of 50 to 1500 μmol/L.

I tested the same potential interfering substances as before (1), but as we have previously demonstrated, I again found no interference with the present method. Bilirubin up to 500 μmol/L and hemoglobin up to 150 μmol/L also do not interfere.

Analytical recovery is complete, ranging from 98.5 to 102% for 50, 250, and 500 μmol of creatinine added per liter to sera containing creatinine concentrations of 75, 200, 450, and 800 μmol/L.

Precision is good: 10 analyses of serum specimens containing 85, 150, 650, 810, and 1240 μmol of creatinine per liter showed within-day CVs ranging from 1.1 to 2.5% and day-to-day CVs from 1.5 to 3%. The results of serum duplicates assayed on the same day with the use of different sample dilutions (0.05–20 mL) of sample averaged 250 (SD 5.5) μmol/L.

To assess the specificity of the method, I compared it with the fully enzymic method of Wahlefeld et al. (4). Results by the proposed method (y-values) and the comparison method (x-values) with specimens from 100 patients whose serum creatinine ranged from 45 to 1480 μmol/L and urinary creatinine from 5 to 14 mmol/L correlated well (r = 0.995, y = 1.031x + 0.061).

Analysis for serum creatinine of 40 women and 60 men, ages 20–50 years, without any former or present signs of renal disease, gave values of 44–85 μmol/L (68 ± 11) for women and 53–102 μmol/L (80 ± 13) for men.

The proposed method is simple, reliable, relatively rapid, and only requires the use of two low-cost, stable reagents at room temperature. It is suitable for use by analysts with little experience, in laboratories with common equipment.

These features recommend its widespread use for "true" creatinine analysis.

References

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Coomassie Blue Method: Work Duplicated
To the Editor:
The recent Letter by Joern and Schmoele (Clin. Chem. 21: 1305, 1981) indicated that the Coomassie Blue test could be successfully adapted to the ABA-100. We have previously published similar results, and also showed that the results compared well with the Ponceau-S dye binding method (1).

Reference

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Serum Ferritin Determination by Enzyme Immunoassay: Importance of Sample Dilution (the "Hook Effect")
To the Editor:
We use a commercial enzyme immunoassay kit for determination of ferritin in serum (NEIA ferritin; New England Immunology Associates, Inc., Cambridge, MA 02138). It is a "sandwich" method, described by Boenisch (7), in which polypropylene tubes coated with antiferritin antibodies are incubated with samples and then with alkaline phosphatase-labeled antibodies. The enzyme activity bound to the tubes is measured with p-nitrophenyl phosphate as the substrate. The calibration curve covers the range 0 to 500 μg/L. Reference values with this technique are 10–70 μg/L for women and 35–230 μg/L for men (2).

We have noted anomalous results for two sera. One was from a 76-year-old woman with icterus by retention, metastases to the liver, biliary stones, and probably primary carcinoma of biliary vesicle. The other was from a 47-year-old woman with carcinoma of the transverse colon and pulmonary metastases. For these two sera, we found higher absorbances for samples diluted five- and 10-fold than for undiluted samples. Values for more highly diluted samples (50- and 100-fold) were much higher than the less-diluted samples (Table 1). The values for the more highly diluted sera from both patients are very pathological, 12690 and 9425 μg/L, and are concordant with the patient's pathology. These same observations were noted with two different lots of reagents. This paradoxically decreased absorbance (as compared with that expected) observed for very high ferritin concentrations is called the "hook effect," and has been noted for two-site immunoradiometric assays for ferritin by several authors (3, 4), but not yet for enzyme immunoassay.

In conclusion, this phenomenon must be taken into account when ferritin is determined by enzyme immunoassay by testing all samples on undiluted serum and at two different dilutions (five- and 10-fold). This precaution obviates the potential error of giving a low value to a patient who has a true pathologically increased concentration of ferritin.

References

Table 1. Effect of Sample Dilution on Results

<table>
<thead>
<tr>
<th>Sample Dilution (fold)</th>
<th>Absorbance at 450 nm</th>
<th>Apparent serum ferritin (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum from Patient One</td>
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<td></td>
</tr>
<tr>
<td>Undiluted</td>
<td>1.098</td>
<td>206</td>
</tr>
<tr>
<td>5</td>
<td>1.827</td>
<td>&gt;2500</td>
</tr>
<tr>
<td>10</td>
<td>1.998</td>
<td>&gt;5000</td>
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<tr>
<td>50</td>
<td>1.320</td>
<td>12 400</td>
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<tr>
<td>100</td>
<td>0.822</td>
<td>12 900</td>
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<tr>
<td>Serum from Patient Two</td>
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<td></td>
</tr>
<tr>
<td>Undiluted</td>
<td>1.970</td>
<td>&gt;500</td>
</tr>
<tr>
<td>5</td>
<td>&gt;2</td>
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