bilibin, hemoglobin, and carotenoids should be minimized.

I analyzed 70 specimens with serum iron concentrations of 30 to 2500 μg/L with the KDA analyzer, using Fenere-S, AMB-610, and ferroine as the chroomogen for iron. The AMB-610 was used as supplied and the Fenere-S and ferroine were made into 5 mmol/L solutions in acetate buffer, pH 4.5, 0.1 mol/L Ascorbic acid, 200 mmol/L, was used as the reducing agent, distilled water as the blank, and an aqueous solution containing 3800 μg of iron per liter as the standard. The procedure was performed as programmed by American Monitor except that the wavelength used with Fenere-S was 590 nm (filter no. 5) and for ferroine 577 nm (filter no. 6).

Values for serum iron obtained with Fenere-S (γ) as the chromogen agreed well with those obtained with AMB-610 (x1) (r 0.96; y 0.91x + 8.9) or ferroine (x2) (r 0.98; y 0.99x2 - 4.0).

The advantage of Fenere-S over ferroine and AMB-610 is that smaller sample sizes may be used with Fenere-S because of the greater molar absorbptivity of the Fenere-S–ferrous complex: I could decrease the sample volume by 35% without losing any sensitivity, a useful advantage in analysis of pediatric and geriatric specimens.

Although this work was done with a KDA analyzer, Fenere-S may be substituted for ferroine in either manual or other automated methods with the same advantages.

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Evaluation of the CIS-Sorin Corticotropin Kit
To the Editor:

We have evaluated the CIS-Sorin (125I) Corticotropin (ACTH) Radioimmunoassay (RIA) kit (distributed by Damon Diagnostics, Needham Heights, MA 02194). The calibrators in this kit are lyophilized normal human plasma pools with synthetic human corticotropin (ACTH) added to give a concentration range of 0–800 ng/L on reconstitution. The radiolabeled and endogenous ACTH are allowed to equilibrate with rabbit antisem to porcine ACTH and the free fraction is separated from the bound by adsorption onto charcoal.

The procedure requires only 100 μL of EDTA-treated plasma, with no extraction or purification step, and involves a 48-h incubation at 2–6 °C. The ratio of calibrator bound to zero-bound (B/B0) ranged from 90% for the 20 ng/L standard to 35% for the 800 ng/L standard. Average initial bindings of bound to total (B/T) were 40%, with nonspecific bindings of 7 to 10%.

The between-run precision for the three different concentrations of controls supplied with the kit by the manufacturer were as follows:

\[
\begin{array}{cccc}
\text{ACTH, ng/L} & \bar{x} \text{ (and SD)} & \text{CV, %} \\
10 & 26 (3.0) & 11.7 \\
10 & 79 (7.6) & 9.7 \\
8 & 361 (13.5) & 3.7 \\
\end{array}
\]

These results were obtained with use of four lot numbers of kits and indicate negligible lot-to-lot variation.

Between-run and within-run precision was determined on a sample from a patient whose plasma ACTH concentration was above normal. The between-run [n = 8; \bar{x} (and SD) = 344 (27.8) ng/L; CV = 8.1%] and within-run [n = 6; \bar{x} (and SD) = 384 (26.9) ng/L; CV = 7.1%] results were quite consistent. These determinations were performed with kits having three different lot numbers.

We assessed linearity by diluting a high-ACTH plasma with plasma containing no detectable ACTH and measuring the percentage analytical recovery at each dilution, based on the measured value of the undiluted sample, with the following results, in ng/L:

<table>
<thead>
<tr>
<th>Dilution Measured Expected M/E. (fold)</th>
<th>M (E)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>465</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>232</td>
<td>232</td>
</tr>
<tr>
<td>4</td>
<td>120</td>
<td>116</td>
</tr>
<tr>
<td>8</td>
<td>55</td>
<td>58</td>
</tr>
</tbody>
</table>

The regression equation was: measured = 1.01 (expected) - 1.00 (r = 0.99), demonstrating excellent linearity.

The package insert indicates that the manufacturer assayed 200 normal subjects, finding a mean of 38 (2 SD = 40) ng/L. These data suggest a highly skewed population distribution. The frequency distribution of these values shows that no normal persons had a plasma ACTH concentration of <10 ng/L, a value that corresponds to the stated limit of analytical sensitivity of the assay. This would indicate a tentative reference interval of 10–78 ng/L.

Samples of EDTA-treated plasma from clinically defined patients (courtesy of Dr. Bernard Kliman, Massachusetts General Hospital, Boston, MA) were assayed. One patient with hypopituitaryism had results of 12 and 13 ng/L for specimens taken before steroid dosage for that day. A similar case had an assay value of 0.0 ng/L. An obese patient with normal cortisol assay values had an ACTH concentration of 30 ng/L at 8 a.m. A patient with Cushing’s disease of pituitary origin had an above-normal result of 108 ng/L at 8 a.m. Samples drawn from a patient with an ACTH-secreting pituitary tumor, Nelson’s syndrome, showed high values of 892 ng/L before therapy with x-ray and 451 ng/L one month after completion of treatment.

These results are within the expected ranges for all patients except for one hypopituitary patient, whose results were borderline low.

These few data seem to indicate a lack of sensitivity at the lower end of the reference range, which would only be important in the evaluation of the relatively rare hypopituitary patient, and other pituitary-function tests would be used to arrive at that diagnosis.

From these preliminary data we conclude that precision is acceptable at three different concentrations of ACTH and that lot-to-lot variation is negligible. The linearity (r = 0.99) and analytical recovery (94.8–103.4%) are good. We believe that the clinical accuracy is acceptable for use in the normal or hyperpituitary patients, although the assay may be too insensitive for measurements in the hypopituitary range. Nevertheless, if relatively low values for both ACTH and cortisol are found, this would distinguish hypopituitarism from Addison’s disease.

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Evaluation of the Roche Modified CPK-CS Kit
To the Editor:

Roche Diagnostics recently introduced a modification of their creatine kinase (CK; EC 2.7.3.2) kit for column separation of isozymes of CK. The new kit (modified CPK-CS) includes elution buffers for CK-MM and CK-MB isoenzymes in which the NaCl concentrations are greater than in the original CPK-CS kit. In both kits, the first buffer (buffer A in the original kit, buffer I in the modified kit) elutes CK-MM, and the second buffer (B in the original kit, II in the modified kit) elutes CK-MB, according to the manufacturer.

To test the accuracy of this claim, we evaluated 96 serum specimens derived from our hospital population, following the protocol exactly for each Roche kit and comparing results for MB eluted in the second buffers, B and II. Measuring the enzymic activity kinetically with the Roche assay, in a Centrifichem centrifugal analyzer (Union Carbide Corp., Pleasantville, NY 10570), we observed a significant increase in apparent