An Automated Dry-Slide Enzymatic Method Evaluated for Measurement of Creatinine in Serum

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We evaluated an automated dry-slide enzymatic method involving creatinine iminohydrolase for measurement of creatinine in serum with the Kodak Ektachem analyzer. The means (and SD) for three commercially available quality-control sera, analyzed during eight weeks, were 9.7 (1.0), 16.6 (0.9), and 61 (2.1) mg/L. The regression equation for 105 samples measured with the Technicon SMAC (x) and Ektachem Analyzers (y) was: \( y = (0.89 \pm 0.007)x + (1.7 \pm 0.3) \) mg/L and for 170 samples measured with the Beckman Astra (x) and Ektachem analyzers (y): \( y = (1.00 \pm 0.005)x - (1.9 \pm 0.16) \) mg/L. Sixty-one samples from renal-transplant patients showed nearly the same agreement. The enzymatic method had no interference from substances that interfere with many Jaffé methods for creatinine, including acetate. The drugs cephalothin and cephotixin did not interfere, but 5-fluorocytosine interfered significantly with creatinine in the Ektachem method. Values for several ketone-positive sera were 5 to 10 mg/L higher by the Astra relative to the Ektachem. Grossly hemolyzed or lipemic samples were analyzed without difficulty. We conclude that this enzymatic method for creatinine in serum has the speed and precision necessary for routine clinical laboratory use and, except for one drug, the method appears to be specific for creatinine.

Additional Keyphrases: multilayer film analysis • discrete analysis • Jaffé reaction

The methods most commonly used in clinical laboratories to measure serum creatinine utilize an alkaline solution of picrate as the colorimetric reagent, a methodology known to be subject to many interferences (1). Modifications such as adsorption of creatinine and extraction (2), dialysis (3), kinetic measurement (4), or enzymatic analysis (5, 6) have been developed, but these either do not entirely eliminate the nonspecific reactions or are not suited for high-throughput clinical laboratory use.

Methods based on multilayered dry-film technology have recently been developed for urea, amylase, bilirubin, triglyceride, and cholesterol (7, 8). We evaluate here another such method, for determination of serum creatinine. The method is based on the enzymatic hydrolysis by creatinine iminohydrolase (EC 3.5.4.21) of creatinine to produce ammonia and N-methylhydantoin, and the selective migration of ammonia through a semipermeable membrane to a layer containing an indicator dye. Another dry-film slide, but without creatinine iminohydrolase, is used for a blank measurement of the endogenous ammonia in serum. The blank-corrected measurement of the ammonia generated from creatinine therefore reflects the creatinine concentration. This new methodology may potentially provide a method specific for creatinine that is fast, precise, and better suited for clinical laboratory use.

Materials and Methods

Creatinine and ammonia slides were used with the Ektachem 100 Analyzer. Both slides and instrument were supplied by Eastman Kodak Co., Rochester, NY 14650. The creatinine cartridges, each containing 50 slides, were loaded after removal from the refrigerator; then the top slide was dispensed to obviate any possibility of a slight effect due to condensation on the uppermost slide. The ammonia cartridges were warmed at room temperature for 30–60 min before loading, and all slides were used.

Two 10-μL drops of samples were automatically deposited, one on a creatinine slide and the other on an ammonia slide. After incubation, the reflectance of each slide was measured and the difference in reflectance between the slides was used to calculate the creatinine concentration. The instrument can do 150 analyses per hour, and results are printed 5.5 min after sampling.

For method-comparison and interference studies, creatinine was measured either in an Astra 8 Analyzer (Beckman Instruments, Inc., Brea, CA 92621) or, in the earlier part of the study, in a SMAC continuous-flow analyzer (Technicon Instruments Corp., Tarrytown, NY 10591). In the Astra 8, creatinine is determined by a kinetic Jaffé method in which creatinine reacts with alkaline picrate; the reaction rate at 26 s is used to calculate the creatinine concentration. The SMAC involves dialysis before measurement of creatinine by the alkaline picrate technique.

Creatinine iminohydrolase, purified from Flavobacterium filamentosum, was used in the Ektachem Creatinine Slide as diagrammed in Figure 1. After spreading, the sample permeates the upper reagent layer where creatinine iminohydrolase generates ammonia from creatinine by the following reaction:

\[
\text{HN N-CH}_3 \xrightarrow{\text{creatinine iminohydrolase}} \text{HN N-CH}_3 + \text{NH}_3
\]

The ammonia diffuses through the semipermeable layer to the lower reagent layer, which contains bromphenol blue as an ammonia indicator. The reflection density at 600 nm is measured at an angle of 45° for both the creatinine and ammonia slides, and this difference is used to calculate the creatinine concentration of the sample.

Four concentrations of calibrators, supplied in lyophilized form by Kodak, were used for each calibration. After the method comparison with the SMAC, the set points for the Ektachem calibrators were modified slightly.

Since this evaluation, Eastman has increased the stability of Ektachem slides for creatinine, so that they may be warmed to room temperature like other slides, and the top slide is used.

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Twice daily, three different lyophilized serum controls were reconstituted and analyzed in duplicate, with at least a 4-h interval between the duplicate analyses. The quality-control sera were: Q-Pak Chemistry Control Serum I (Hyland Diagnostics, Division of Travenol Laboratories, Inc., Deerfield, IL 60015), and Assure Level I and Assure Level II Clinical Chemistry Serum Controls (General Diagnostics, Division of Warner-Lambert Co., Morris Plains, NJ 07950).

Some sera were tested for the presence of acetooacetate with Acetest (Ames Company, Division of Miles Laboratories, Inc., Elkhart, IN 46514). This test is quite sensitive to acetooacetate (detecting as little as 50 mg/L), one tenth as sensitive to acetone, and does not detect β-hydroxybutyrate.

For the interference studies, compounds were added in powder form to a human serum pool having a creatinine concentration of about 20 mg/L. Sources of the compounds were: bilirubin, purified from bovine gall stones (Sigma Chemical Co., St. Louis, MO 63178); moxalactam, cefamandol, and cephalothin (Eli Lilly and Co, Indianapolis, IN 46285); cefazolin and cimetidine (Smith Kline and French Laboratories, Philadelphia, PA 19101); cephalaxin (Dista Products Co, Indianapolis, IN 46285); 5-fluorouracil, 5-fluorouracil (Roche Laboratories, Nutley, NJ 07110); acyclovir (Burroughs-Wellcome Co, Research Triangle Park, NC 27709); ceftotaxime (Hoechst-Roussel Pharmaceuticals, Somerville, NJ 08876); cephapirin (Bristol Laboratories, Syracuse, NY 13201); and amphotericin B (Squibb and Sons, Inc, Princeton, NJ 08540).

Least squares statistics were determined and interpreted as recommended by Davis et al. (9).

**Results**

As shown in Table 1, precision was improved when calibration was done less frequently. A factor limiting precision to about 1 mg/L (SD) may be the use of two slides for each measurement, which adds a component of variation that is more apparent at lower creatinine concentrations.

Initially, we analyzed 105 sera for creatinine with both the SMAC and the Ektachem. The data indicate that at concentrations of creatinine exceeding about 50 mg/L, the results from the Ektachem were clearly lower than those from the SMAC. This resulted in a regression equation of: Ektachem = (0.89 ± 0.007) SMAC + (1.7 ± 0.3) mg/L (r = 0.997).

The principal difference between creatinine results by the Astra and Ektachem analyzers was a fixed bias with results by the Ektachem about 2 ± 0.2 mg/L lower (p < 0.01). The means by each method also indicated this (p < 0.01): Astra 23 ± 3 mg/L; Ektachem 21 ± 3 mg/L. The regression equation was: Ektachem = (1.00 ± 0.005) Astra - (1.9 ± 0.2) mg/L (n = 170; r = 0.998). Another comparison of 61 samples from renal-transplant patients gave a similar regression equation: Ektachem = (0.99 ± 0.01) Astra - (0.7 ± 0.4) mg/L.

Glucose and fructose at 55 mmol/L caused a slight positive interference with the picrotate method and a slight negative interference with the enzymatic method. In a study of samples from patients, 32 samples with glucose concentrations ranging from 0.45 to 11.0 g/L were analyzed by both methods. As shown in Figure 2, at higher glucose concentrations, the difference between results by the two methods tended to become larger. The added bilirubin did not detectably interfere with either method in concentrations up to 160 mg/L. Samples from patients with above-normal bilirubin concentrations often had variable differences in creatinine measurements and, as shown in Figure 3, the total bilirubin concentration did not correspond to the magnitude of difference between creatinine methods. To determine if this difference was possibly related to protein binding, we measured albumin. Most of the samples with the greatest differences in creatinine had albumin concentrations of less than 30 g/L, but there were some exceptions. The sample with the greatest difference between methods was negative for ketones (Acetest).

**Table 1. Precision of Creatinine as Measured with the Ektachem Analyzer, with Different Frequencies of Calibration**

<table>
<thead>
<tr>
<th>Material</th>
<th>n*</th>
<th>mg/L</th>
<th>SD</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. From Dec. 22 to Mar. 18: Weekly calibration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q-Pak I</td>
<td>181</td>
<td>9.6</td>
<td>1.2</td>
<td>14</td>
</tr>
<tr>
<td>Assure Level I</td>
<td>192</td>
<td>16.4</td>
<td>1.3</td>
<td>7.9</td>
</tr>
<tr>
<td>Assure Level II</td>
<td>192</td>
<td>61.4</td>
<td>2.2</td>
<td>3.6</td>
</tr>
<tr>
<td>B. From April 12 to June 3: Calibration on April 12, 20, May 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Q-Pak I</td>
<td>145</td>
<td>9.7</td>
<td>1.0</td>
<td>10</td>
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<tr>
<td>Assure Level I</td>
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<td>16.6</td>
<td>0.9</td>
<td>5.4</td>
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<tr>
<td>Assure Level II</td>
<td>152</td>
<td>61.0</td>
<td>2.1</td>
<td>3.4</td>
</tr>
</tbody>
</table>

* Daily duplicate analyses, at least 4 h apart. All data included.
For creatinine as measured by the two picrate methods, 1 mmol/L acetoacetate or pyruvate increased creatinine by about 5 mg/L, and acetone or ascorbate at 1 mmol/L increased it by 1 mg/L. None of these compounds had a detectable effect on the enzymatic method. Hippurate up to 3 mmol/L did not interfere with any method. Results for several samples that were positive for acetoacetate (Acetest) differed markedly between creatinine methods (Table 2). Grossly lipemic or hemolyzed samples were not a problem for the dry-film method, but occasionally were troublesome when analyzed in the Astra Analyzer, as shown in Table 2.

One hundred milligrams of either cephalothin or cefoxitin per liter increased the results for apparent creatinine in the Astra 8 by about 5 mg/L, but the Ektachem method showed no interference by any of the cephalosporin antibiotics we studied. Although values for most samples were lower by the enzymatic method, a sample from a patient who was receiving 5-fluorocytosine (Ancobon) and amphotericin had a markedly higher apparent creatinine by the enzymatic method (Table 2). When we added these drugs to a serum pool, 100 mg of 5-fluorocytosine per liter increased creatinine as measured by the enzymatic method by about 50 mg/L; amphotericin was without effect. Several other drugs added to give concentrations from 100 to 500 mg/L did not detectably interfere with either method: cephalaxin, ceftaxime, cepahaprin, cefazolin, moxalactam, cimetidine, and acyclovir.

A sample that differed by 7 mg/L between methods was from a patient scheduled for pancreactectomy and partial gastrectomy for cancer, and he was receiving total parenteral liquid nutrition. The liquid diet had been changed from high carbohydrate to high amino acid content (Aminocen) on the twelfth day of the month. Samples collected during the high carbohydrate diet were negative for ketones and typically differed little between methods, while those during the high amino acid diet differed by 3 to 7 mg/L (Figure 3). Samples from day 13 to day 15 tested positive for ketones, and we believe that amino acid metabolites caused the higher values obtained with the Astra. The attending physician considered that there was no evidence of change in renal function during this time: urine volume remained good and values for urea changed only slightly, probably because of the amino acid diet.

Discussion

We found substantial differences between methods in the case of only a few patients. Values for most samples were typically about 2 mg/L lower by the enzymatic method. Some enzymatic methods for creatinine are reported to be imprecise (10), but precision by the Ektachem technique is good, comparable to that with automated creatinine assays in which alkaline picrate is used (11). Apparently the slides, and the optics and electronics of the instrument, are sufficiently stable to allow calibration at monthly intervals. We find that analysis of quality-control material is necessary no more than once for each cartridge. In the dry-slide technology a separate disposable pipet and slide are used for each sample, thus eliminating any drift due to carryover or contamination of cuvettes.
Sources of interferences with Jaffé methods, such as acetocacetate and pyruvate (4) and cephalosporin antibiotics (12, 13) have been eliminated in this enzymatic method. Glucose interferes slightly and negatively with the Ektachem slide, about equivalent to the positive interference by glucose in the Astra. The interference is noticeable for glucose concentrations \( \geq 5.0 \text{ g/L} \) but should not be a problem in clinical diagnosis, unless glucose concentrations are changing quickly and extremely.

The only significant interference observed with the Ektachem procedure was by the antibiotic 5-fluorocytosine, usually given with amphotericin for cryptococcal infections. Although 5-fluorocytosine is used much less often than the cephalosporins, its interference is sufficiently large to make it necessary to notify the laboratory when the sample is from a patient receiving this drug. The mechanism of this interference is probably that the 4-amino group of the drug is converted to free ammonia. Amphotericin and several other drugs with an amino group did not interfere.

Interference with the alkaline picrate reaction by bilirubin can apparently be minimized by appropriate reagents and instrumentation (14, 15) such as used for the Astra 8. As previously discussed in detail (4), above-normal bilirubin may be only indirectly associated with an interference in some Jaffé methods. In our study, most of the icteric samples with the greatest difference between methods had low albumin concentrations. Perhaps the interfering form of bilirubin has less effect when it is bound to albumin. There are several forms of bilirubin in serum (15), and a better understanding of them may explain the interference sometimes associated with samples with a high value for total bilirubin. Also, the purified bilirubin added to our serum pool may not be a form of bilirubin that interferes in kinetic Jaffé methods.

The increased difference between creatinine measurements in samples positive for ketones, including those from the patient changed to a high amino acid diet, indicate that the enzymatic technique can be more reliable than the kinetic Jaffé method under certain circumstances. Conditions that increase the concentrations of ketones—such as diabetic ketoacidosis, starvation, renal insufficiency, or hepatitis—may be especially suited to measurement of creatinine enzymatically.

We thank Eastman Kodak Co. for providing the slides and for use of the instrument.

References