assay was observed in patient serum samples with increased bilirubin, rheumatoid factor, immunoglobulins, triglycerides, or hemolyzed sera.

Serum samples from 131 subjects were chosen by their T concentration to cover the Elecsys T assay analytical range (0.069–52 nmol/L) and also to include T at clinically relevant concentrations. Among these subjects we included 10 hypogonadal children, 32 patients with prostate cancer after antiandrogen therapy, 28 hirsute women, and 14 females with polycystic ovarian syndrome. These samples were tested by the Elecsys, and the results were compared with those obtained with the previous routine RIA assay of our laboratory (Spectria-Orion Diagnostica). The relation between the ECLIA and the RIA T is shown in Fig. 1B (11). The regression analysis showed a slope of 0.996, an intercept of −0.324, and a correlation coefficient of \( r = 0.977 \) (\( r^2 = 0.986 \); \( S_{\text{obs}} = 1.385 \)). The 95% confidence interval [IC 95% (\( b \), 0.957–0.998)] showed that the slope was slightly different from 1.0, but the 95% confidence interval of the intercept [IC 95% (\( a \), −0.450–0.216)] was not statistically different from zero. We also evaluated whether the results obtained by the two methods could be interchangeable in two selected groups of patients. The first group (G1) consisted of 32 males with prostate cancer under treatment with antiandrogen therapy. The second (G2) included 28 hirsute women and 14 females with polycystic ovarian syndrome. We calculated the linear regression for both groups, finding a better correlation for the males (\( r = 0.798 \)) than for the females (\( r = 0.696 \)). These differences cannot be attributed to differences in the ranges of values for men and women, and may reflect interference from binding globulins and also cross-reactivity with structurally related compounds in the T immunoassays. We did not have the opportunity to validate this T assay with a reference method such as gas chromatography-mass spectrometry (7).

We evaluated the agreement between the ECLIA and the RIA for classification, using a reference interval of 0.694–2.776 nmol/L in women. All the men in G1 were expected to have T <0.9 nmol/L (3). The concordances of the two methods were 96% for G1 and 80% for G2. Taking both groups together (74 subjects), the two methods provided the same classification in 69 (89%) of the cases.

The Elecsys 2010 assay for T in serum is appealing for use in the routine laboratory. The assay is automated and rapid, giving the first result in 18 min, with 58 additional results in the following hour. The calibration curve was stable for at least 2 months, in contrast to the RIA for T, which requires a new calibration curve in each protocol. In addition, the T assay allows the use of nonradioactive reagents and offers an extended range, allowing a minimal number of sample dilutions. The Elecsys T assay showed a high degree of reproducibility; linearity and comparison studies were satisfactory and we found no carryover problem. It clearly separated hyperandrogenic women from healthy ones, and hypogonadal or androgen-blocked patients from healthy men. These characteristics tend to give quite a good diagnostic potential and reinforce the T assay as a cost-effective front-line androgen function test.

We thank Boehringer Mannheim, Barcelona, Spain, for supplying the T assay reagents free of charge.

References

Single Dilution for Urine Assays on the Vitros 250 or 700 Analyzers, Nadine Raby, Christine Bonneau, S. Gillier, Jaroslava Le, Robert Granouillet, Jacques Frey, and Annette Chansom (Laboratoire de Biochimie, Faculté de Médecine, 15 rue Ambroise Paré, 42023 Saint-Etienne Cedex 2, France; *author for correspondence: fax 33-4-7742-14-89, e-mail frey@univ-saint-etienne.fr)

We previously verified (1) the suitability of slide chemistry (2) with the Vitros 250 or 700 analyzers (formerly Ektachem 250 or 700, Johnson & Johnson) for analysis of urine samples. The sodium, potassium, urea, creatinine, phosphorus, and uric acid measurements, however, required the preparation of sample dilutions, which dramatically reduced analysis speed. The approach was further complicated by the fact that the diluting agent for sodium and potassium was the Urine Electrolyte Diluent (Johnson & Johnson), which could not be used for the other tests, which required dilutions with water.

The present study was therefore carried out to attempt to standardize the dilutions with a single diluent and a single dilution. Because the diffusion of the sample in the spreading layer of the slides is important in slide technology, we tried an aqueous diluent selected with added surfactant. Preliminary studies (not shown) showed the possibility of ion determination with a Beckman diluent.
(wash solution, ref. 668601) without disturbances of the creatinine measurement. Therefore, to obtain the same surface tension with an aqueous solution of more defined composition, we tried a 0.15 g/L solution of Tween 20 (Merck, ref. 822184). This solution could be stored for 1 week. Because the dilution for sodium and potassium was 1:5, the dilution for urea and creatinine was 1:21, and the dilution for phosphorus and uric acid was 1:11 in the original system, we used a single dilution of 1:8 as a compromise, and then collected the following data.

The data obtained in two laboratories were compared. Laboratory 1 was equipped with a Vitros 250, which diluted samples automatically, whereas laboratory 2 used the Vitros 700 with manual dilutions. Tubes containing 7 mL of diluent were prepared in advance, and 1 mL of urine was added to each just before analysis in the practice of laboratory 2.

Briefly, Vitros slides for Na\(^+\) and K\(^+\) used direct potentiometry with valinomycin for K\(^+\) and monensin for Na\(^+\) (3); urea slides used urease to generate ammonia, Table 1. Linearity limits.

<table>
<thead>
<tr>
<th></th>
<th>Multiple dilution method</th>
<th>Single dilution method</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Sample dilution</td>
<td>Limits, mmol/L</td>
</tr>
<tr>
<td>Na(^+)</td>
<td>1:5</td>
<td>5–250</td>
</tr>
<tr>
<td>K(^+)</td>
<td>1:5</td>
<td>2.5–175</td>
</tr>
<tr>
<td>Urea</td>
<td>1:21</td>
<td>15–900</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1:21</td>
<td>0.084–30.6</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>1:11</td>
<td>1.76–46.2</td>
</tr>
<tr>
<td>Uric acid</td>
<td>1:11</td>
<td>0.327–11.12</td>
</tr>
</tbody>
</table>

\(^a\) Limits shown in the manufacturer’s text methodology manual.
\(^b\) Limits determined with samples previously diluted to obtain a large concentration scale.

Fig. 1. Difference plots between use of a single 1:8 dilution (with 0.15 g/L Tween 20) and standard multiple dilutions per manufacturer’s directions (1:5, 1:11, and 1:21, depending on the analyte).

M, mean of the differences; SD, standard deviation of the differences. The mean value of two methods is plotted on the x-axis. (○), data points for laboratory 1 (automatic dilutions with Vitro 250); (––––––), mean and 2-SD values for laboratory 1; (●), data points for laboratory 2 (manual dilutions with Vitros 700); (———–), mean and 2-SD values for laboratory 2.
which reacted to form a color with merocyanine dye (4); creatinine was hydrolyzed to creatine, which in a reaction cascade, led to production of hydrogen peroxide that was measured by triaryl imidazole leuco-dye (4); slides for phosphorus used the formation of an ammonium-phosphomolybdate complex and its reaction with \( p \)-methylvanilinophenol sulfate (5); and uric acid was determined by the uricase peroxidase method (6). The specific urine calibration was used.

The linear regression between the multiple dilution and single dilution methods was checked for urea, creatinine, phosphorus, and uric acid. In the case of sodium and potassium, the slopes were different from 1.0, and the intercepts were different from 0 (\( P < 0.001 \)). Corrections were carried out by introduction of slope and intercept values established with the flame photometry reference method (Eppendorf apparatus) for calibration of each new batch of slides. The linearity limits were modified when a single dilution was used, as shown in Table 1. These new limits were determined by successive dilutions of samples with high concentrations of analytes.

The imprecision was checked by comparison of multiple dilutions and single dilution with the Vitros 250 (laboratory 1) and Vitros 700 (laboratory 2), using control urine (Biotrol, A02262). The within-day CVs of the two methods (10 determinations) in the two laboratories were \( \leq 1\% \). The mean values (±SDs) for different control ranges, respectively, in laboratories 1 and 2 were as follows: Na (127.3 ± 1.49 mmol/L; 89.3 ± 0.72 mmol/L), K (72 ± 0.67 mmol/L; 31.5 ± 0.3 mmol/L), urea (279.5 ± 3.19 mmol/L; 166 ± 2.06 mmol/L), creatinine (9.28 ± 0.09 mmol/L; 3.67 ± 0.04 mmol/L), phosphorus (17.91 ± 0.23 mmol/L; 2.65 ± 0.018 mmol/L), and uric acid (2.18 ± 0.02 mmol/L; 0.60 ± 0.006 mmol/L).

The day-to-day CVs (30 determinations in laboratory 1 and 50 determinations in laboratory 2 of control urine) were <2% for all analytes. One determination was carried out each day during a 1-month period in laboratory 1 and a 2-month period in laboratory 2. The freeze-dried control urine was reconstituted each week and stored at 4 °C. Every day, a fresh dilution was made. Laboratories 1 and 2, respectively, obtained the following results with a different lot in each laboratory: Na (122.6 ± 2.45 mmol/L; 121.6 ± 2.49 mmol/L), K (70.88 ± 1.10 mmol/L; 60.1 ± 0.95 mmol/L), urea (280.1 ± 5.83 mmol/L; 220 ± 2.74 mmol/L), creatinine (9.45 ± 0.20 mmol/L; 7.84 ± 0.09 mmol/L), phosphorus (17.13 ± 0.30 mmol/L; 15.85 ± 0.20 mmol/L), and uric acid (2.17 ± 0.04 mmol/L; 1.94 ± 0.02 mmol/L).

The agreement between the single dilution system and the standard multiple dilution system was assessed by analyzing patient urines by the two methods with both automatic (Vitros 250) and manual (Vitros 700) dilutions. Differences from the mean were calculated (7). Fig. 1 shows the differences between methods for each analyte. The observed differences were small compared with the reference ranges. It is therefore clear that a single dilution of urine with aqueous Tween 20 can be used for assaying sodium, potassium, urea, creatinine, phosphorus, and uric acid without large error. Although this improved method is not yet endorsed by the manufacturer, it makes urine analysis on the Vitros apparatus much faster with or without automatic dilutions.

References

Optimized Procedure for DNA Isolation from Fresh and Cryopreserved Clotted Human Blood Useful in Clinical Molecular Testing, Luis A. Salazar, Mario H. Hirata, Selma A. Cavalli, Marcos O. Machado, and Rosario D.C. Hirata (Faculty of Pharmaceutical Sciences, University of Sào Paulo, CEP 05508-900, Sào Paulo, SP, Brazil; * address correspondence to this author at: Dept. of Clinical and Toxicological Analysis, Faculty of Pharmaceutical Sciences, University of Sào Paulo, Av. Prof. Lineu Prestes, 580, CEP 05508–900, Sào Paulo, SP, Brazil; fax 011-813-2197, e-mail mdchirta@usp.br)

In the routine clinical laboratory, large amounts of uncultured blood are collected and the blood clot usually is discarded. In molecular biology, cells from EDTA-anticoagulated or acid-citrate-dextrose-anticoagulated peripheral blood are used as sources of DNA (1–3). After leukocyte isolation, most procedures utilize enzymatic cell digestion, followed by extraction with hazardous organic solvents (phenol-chloroform) and precipitation with ethanol (4, 5). To minimize the volume of blood collected for laboratory tests, several authors have developed methodologies to isolate DNA from blood clots (4–8). However, the techniques may be difficult or impractical and may require slicing of the clot with scalpels or other sharp instrument, exposing laboratory personnel directly to contaminated blood (4, 7). Other techniques are time-consuming, using many chaotropic reagents, enzymes, RNA-removal steps, or large volumes of samples and reagents not suitable in the clinical laboratory (4–8).

We have optimized a nonenzymatic, nontoxic procedure for efficient DNA extraction from fresh and cryopreserved clotted blood.

Blood samples were obtained from 24 unrelated individuals who had given informed consent. We compared 10 paired samples of EDTA-anticoagulated blood and fresh blood clot and studied 14 samples of cryopreserved