Automated Determination of Urinary Na⁺, K⁺, Chloride, Inorganic Phosphate, Urea, and Creatinine without Sample Dilution, with the "RA-XT"

Paul D. Fees,1 Monique Pressac,1 François Braconnier,2 and Pierre Aymard1

We describe how concentrations of chloride, urea, inorganic phosphate, and creatinine in urine can be measured directly, without manual sample dilution, in a discrete analyzer (the Technicon "RA-XT"). These methods were accurate for concentrations of chloride up to 280 mmol/L, urea up to 500 mmol/L, inorganic phosphate up to 50 mmol/L, and creatinine up to 30 mmol/L. CVs are <3% and results correlate well with those obtained by continuous-flow analysis (SMA-II). All these reagents are stable at room temperature for three weeks. Analyses are easy to perform and infrequent calibration is required.

Additional Keyphrases: Technicon RA-XT · random-access analysis · continuous-flow and discrete analysis compared · direct urinalysis

The RA-XT analyzer (Technicon Instruments Corp., Tarrytown, NY 10591) is suited for clinical use in measuring Na⁺, K⁺, chloride, and inorganic phosphate in plasma, but the chief difficulty with similar assays of urine is the unpredictable concentrations of these electrolytes, which may necessitate use of a number of different dilutions so that one or more will be within the analytical range of the method.

We adapted and evaluated methods for the RA-XT analyzer for measuring sodium, potassium, chloride, inorganic phosphate, urea, and creatinine in urine, directly, without manual sample dilution. We compared results obtained by these new methods with those obtained by continuous-flow methods with the SMA-II.

Materials and Methods

Specimens. Twenty-four-hour urine specimens from 70 patients were collected without added preservative, and centrifuged. We determined precision by assaying pooled specimens of urine containing high, medium, and low concentrations of analytes.

For the linearity study we used Technicon Urine Calibrator (Na⁺, 250 mmol/L; K⁺, 100 mmol/L) and an aqueous solution for chloride inorganic phosphate and creatinine. These solutions were diluted to 6.25, 12.5, 50, 80, and 100% of their original concentration.

Assay methods. All reagents were from Technicon, 95331 Domont, France. With the RA-XT, Na⁺/K⁺ were analyzed by use of an ion-selective electrode. The method for chloride measurement is based on the reaction between ferric perchlorate and Cl⁻. For urea, the reaction with o-phthalaldehyde and N-(1-naphthyl)ethylenediamine is measured by the quadratic kinetic method. For determination of creatinine, we used the Jaffé reaction as modified by Kroll et al. with a low sodium hydroxide concentration. For the inorganic phosphate measurement, we used a direct ultraviolet method that requires only a single reagent addition: 2 μL of urine diluted 200-fold with molybdate solution; the resulting complex is measured at 80 nm. All these reagents are stable at room temperature for at least three weeks.

In the SMA-II, for chloride we used TPTZ (mercuric-2,4,6-tri(2-pyridyl)-5-triazine); for urea, the diacetyl reagent; for creatinine, picric acid and NaOH reagents from Technicon; and for inorganic phosphate, a classical molybdenum-blue reaction. In this instrument all assays are performed on dialysates.

Calibration. The RA-XT ion-selective electrode was calibrated every 4 h with two solutions from Technicon. For chloride, creatinine, and inorganic phosphate, we calibrated with an aqueous solution containing 12.5 mmol of phosphorus, 129 mmol of chloride, and 8.84 mmol of creatinine per liter. We calibrated urea with appropriate dilutions of a standard urea nitrogen stock solution, 500 mmol of urea per liter. These solutions are stable at room temperature for one month. Chloride, urea, creatinine, and inorganic phosphate are calibrated once a week. For the SMA-II, we used calibrators from Technicon.

Statistical analysis. We analyzed the results by analysis of variance and standard linear regression (arbitrarily choosing to use the SMA-II results as the independent variable).

Results

Precision. The within-assay precision for pooled patients' urines and the between-assay precision for quality-control material are shown in Table 1.

Linearity. RA-XT responses varied linearly with electrolyte, chloride, creatinine, and inorganic phosphate concentrations (Table 2). For urea, the relationship of absorbance

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Discussion

Na⁺/K⁺ measurement was satisfactorily precise for both. Linearity was acceptable and the linear ranges suffice for clinical use (6). Results by all methods from the RA-XT correlated well with those from the SMA-II. Despite high concentrations of Na⁺/K⁺, we detected negligible carryover with the ion-selective electrode assays, and we conclude that the system is satisfactory for both serum and urinary electrolyte measurements. After a urinary analysis, only three calibration cycles are necessary for Na⁺/K⁺ measurements in serum or plasma samples.

For chloride analysis, the ferric perchlorate method, described by West and Coll (7) and recently automated on a centrifugal analyzer (2), completely eliminates mercury from the reagent. The absorbance-concentration relationship is linear and the reaction is more specific for chloride than are most others. Thus, when we calibrated with an aqueous solution containing 129 mmol of chloride per liter, we obtained the best correlation between patients' samples.

For determining inorganic phosphate, the method quantifies the unreduced phosphomolybdate heteropolyacid at 380 nm and the standard curve is linear to at least 250 mmol/L, given the 200-fold dilution of sample with molybdate solution. Addition of detergent prevented protein precipitation and turbidity. The correlation for the inorganic phosphate seems to be poor at concentrations exceeding 20 mmol/L, but it is evident that in the SMA-II the relation of reduced phosphomolybdate to measured concentration starts to become nonlinear at this value.

For determination of urinary urea nitrogen, we used the kinetic method described by Jung et al. (3). We increased...
the range of the reaction by halving the final concentrations of o-phthalaldehyde and naphthylethlyenediamine in the final mixture. With these lower concentrations, useful ranges can be obtained with a 4-μL sample and an incubation time of 4 min 30 s. Because of nonlinearity, for concentrations >250 mmol/L we used a quadratic kinetic method with multipoint calibration. Urea concentration and absorbance at 500 nm are related throughout a wide range of concentrations, 0 to 500 mmol/L, without dilution of the sample and with good sensitivity, 1.6 A for 500 mmol of urea nitrogen per liter. Results by this method correlate well with those by the diacetyl method involving dialysis, and there is no interference from ammonia.

For determination of urinary creatinine, an aqueous standard is preferred because it more closely resembles urine. We obtained results that compared well with those of the continuous-flow method. We used the "low-hydroxide" method (4), which is rapid (no urine dilution), cost-effective, and easy to perform. For all these analyses, interferences are those described by the authors. So high concentrations of cations such as Li⁺, Ca²⁺, and Mg²⁺ did not interfere with measurements of Na⁺ or K⁺ (6). Likewise, high ammonia values did not interfere with the RA-XT ion-selective electrode for Na⁺ and K⁺ measurements.

For determination of urinary creatinine, the potential interferents—protein, glucose, acetone, bilirubin, urea, ascorbic acid—did not affect results (4). The specificity of the reaction for urea is comparable to that for the diacetyl reaction except when sulfa compounds are present, but these are rarely used at our hospital. Citrulline and β-ureidopropionic acid (3), which interfere with the present reaction, are rarely present in abnormally high concentrations in urine.

For urinary chloride, interferences by bilirubin and hemoglobin are eliminated by the bichromatic procedure and interference by bromide is negligible in this method. As reported by Law and Ertinghausen (2), ascorbic acid, salicylate, cyclophosphamide, digoxin, t-dopa, and caffeine did not produce any interference at therapeutic dosage concentrations.

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References

Interference of Coumarin Therapy with the "Heptest" Owing to Declining Prothrombin Concentrations
Johan Fischer, Bert Verbruggen, Hans Wessels, and Clemens Haanen

The Heptest kit (Haemachem, Inc., St. Louis, MO) for quantifying heparin in plasma is based on heparin-mediated inhibition of factor Xa, resulting in prolongation of clotting time. In 19 of 55 plasma samples obtained from 32 patients concurrently receiving coumarin and heparin, Heptest results exceeded true heparin values by more than 0.2 int. unit/mL; four samples showed a deviation exceeding 0.4 int. unit/mL. We show here that these deviations are caused by coumarin-induced decreases of plasma prothrombin. This problem can be circumvented by adding purified prothrombin or normal plasma to the assay mixture.

Therapy with heparin requires its precise and accurate quantification in plasma, to avoid both inadequate antithrombotic treatment and bleeding complications. Several assay procedures have been developed (reviewed in ref. 1) for determining its concentration in plasma.

The "Heptest," a recently introduced commercial test, is based on the inhibition of factor Xa activity mediated by heparin, ultimately resulting in prolongation of plasma clotting time (2, 3). All relevant clotting factors except for antithrombin III and prothrombin are supplied with the kit. Use of Heptest should be restricted to the determination of heparin concentrations in plasma, because in vitro measurement of anticoagulant activity of both heparin and oral anticoagulants cannot be considered to represent the in vivo anticoagulating status. Heparinization is usually accompanied by therapy with oral anticoagulant (e.g., coumarins) for long-term treatment after cessation of heparin therapy. A decrease in the concentration of prothrombin—one effect of oral anticoagulants—may interfere with proper measurement of heparin concentrations by the Heptest. In the present study, we compared a chromogenic assay with the Heptest to determine the heparin concentration in plasma samples from patients receiving antithrombotic therapy.