Measurement of Urinary Chloride with the Kodak Ektachem 400

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Urinary chloride can be measured simply, precisely, and accurately with the Ektachem 400 clinical chemistry analyzer. Because the coated Nim technique used in this instrument requires the presence of a protein matrix, we added a protein-based calibrator to the urine samples to overcome the low protein concentration in these specimens. Its advantages over a coulometric titration method (Astra analyzer) include the extension of the range of linearity to lower concentrations and the absence of interference from prednisone metabolites in urine. A disadvantage is the negative bias interference of allopurinol.

Additional Keyphrases: multilayer film analysis · colorimetry · coulometric titration compared · alkalinemia

Accurate measurement of chloride in urine is especially important in cases of persistent metabolic alkalosis, to distinguish patients with hyperadrenocorticism or hyperaldosteronism from patients with decreased chloride intake or excessive chloride loss, a differentiation crucial for management of the patient (1). While patients with severe gastrointestinal loss of chloride respond promptly to saline infusion, patients with chloride-resistant metabolic alkalosis—as in Bartter's syndrome, hyperaldosteronism, or Cushing's disease—will not respond.

The manufacturer does not provide a procedure for measuring urinary chloride in the Ektachem 400 multiple discrete analyzer (Eastman Kodak Co., Rochester, NY 14650). We have developed a simple modification of their method for measuring chloride in serum, which overcomes the problems of low protein concentration in urine samples and provides accurate measurement of chloride in samples with chloride concentrations far below the lower range of linearity of the instrument. We assessed the validity of the new method by comparison with our previous method, in which we used an Astra-8 multiple discrete analyzer (Beckman Instruments Inc., Fullerton, CA 92634).

Materials and Methods

Reagents. We used Kodak Ektachem calibrator no. 2 to modify the chloride and protein content of the urine specimen, reconstituting the lyophilized calibrator with Kodak Ektachem calibrator diluent. For analytical-recovery studies we used NERL electrolyte standards (New England Reagent Laboratory, East Providence, RI 02914).

Specimen collection. Random (untimed) urine specimens were collected without preservative, covered, and stored at 4°C until tested the same day with both instruments.

Procedure with the Ektachem. 1. Prepare "concentrated calibrator" by adding 2.5 mL of calibrator diluent (instead of the usual 3 mL) to the lyophilized contents of the calibrator no. 2 and gently mix for 30 min to dissolve completely. This reconstituted solution can be used for up to 48 h if stored at 4°C. Less than the usual amount of diluent is added so that the protein concentration of the mixture will be closer to that of serum.

2. Mix 100 μL of distilled water with 100 μL of calibrator fluid and analyze this sample with the Ektachem, using the serum-chloride standard curve. Record result as the "urine chloride correction factor" for calculations. Perform this calibration once every day and with every new bottle of the calibrator.

3. Mix 100 μL of urine sample well with 100 μL of calibrator fluid and analyze this with the Ektachem, again using the serum-chloride curve (range of linearity 50–170 mmol/L). Record the result as the "actual reading."

4. Calculate the chloride content of the sample according to the equation

\[ \text{Cl, mmol/L} = \frac{(AR - CF) \times 2}{n} \]

where AR is the actual reading, CF is the correction factor, and 2 is the dilution factor.

Comparison procedure. Chloride was also measured in all samples according to the manufacturer's instructions for chloride content in the Beckman Astra (range of linearity 15 to 400 mmol/L).

Method-comparison study. We analyzed 100 specimens by both methods. Comparison was based on Model II (2) linear regression. The patient's clinical history was obtained for all specimens showing values outside of the 95% confidence limits for the linear regression.

Precision study. Day-to-day reproducibility was determined by duplicate analyses of random specimens in two separate runs and by analysis of an in-house urine pool analyzed once per run.

Recovery study. Aqueous sodium chloride solution was added to aliquots of urine samples of known chloride concentration to give final concentrations between 48 and 188 mmol/L. Each sample was analyzed twice.

Statistical methods. To examine linearity we used a standard Model I least squares linear regression. For method comparison we used Model II linear regression (2).

Results

Figure 1 illustrates the comparison between results by coulometry (Astra) and multilayer film analysis (Ektachem). The linear regression equation is:

\[ \text{Ektachem} = (1.05 \times \text{Astra}) + 3.55 \text{mmol/L} \]

n = 78; r = 0.970; SD of residual error = 6.3
For some specimens the actual chloride concentration was not accurately measured in the Astra because the lower limit of linearity for this instrument is 15 mmol/L. These results were therefore not included in Figure 1 or the statistical calculations.

The between-run CV of the proposed Ektachem is 2.8% at 109 mmol/L as calculated for duplicate specimens from patients, and 4.3% at 83.3 mmol/L for quality-control material once per day for 30 days.

We evaluated the lower limit of linearity of the new method by analyzing serial dilutions of a 100 mmol/L sodium chloride solution down to 3.1 mmol/L. Linear regression results are: measured chloride = 0.985 * nominal chloride + 0.589, r = 0.999, n = 7. We conclude that this method gives dependable results down to 5 mmol/L.

The mean analytical recovery of added chloride was 101.8% ± 1.18% (SEM).

For two groups of patients, the two methods gave discrepant results, i.e., points more than three standard deviations of the residual error from the regression line. Patients in the first group were either on a low salt diet (2–4 g of NaCl per day) or had hypochloremia and metabolic alkalosis caused by severe vomiting and diarrhea. We noticed that the Astra values were higher than expected for such patients and that results for urinary chloride measured with the Ektachem were more consistent with the patients’ clinical status (3). All of these patients were receiving prednisone treatment for various underlying diseases. The second group consisted of patients showing lower than expected values for urinary chloride with the Ektachem 400 method; all of these patients were being treated with allopurinol.

Discussion

Correlation of results for urinary chloride between the proposed Ektachem method and the comparison method is excellent except for the two groups of patients mentioned. A possible explanation for the positive bias of the Astra chloride method for patients taking prednisone is the excretion of metabolites of prednisone as water-soluble sulfates or glucuronic acid conjugates (4). Steroidal sulfates interfere positively with the Astra method (5). Allopurinol has been implicated as a major negatively interfering substance with the Ektachem serum chloride method (6) in renal failure patients, and this may explain the lower-than-expected values for urinary chlorides with the proposed method in patients taking allopurinol.

The method described here is easy, accurate, and precise. The extended lower limit of linearity is a major advantage of the Ektachem method over the Astra because it is well below the clinical decision level of 10 mmol/L (7).

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References