**Letters**

**Improved Spectrophotometer Evaluated**

To the Editor:

In a recent issue of Clinical Chemistry, notice was taken of the new IL 751 dual-channel Atomic Absorption Spectrophotometer, and a question was implied (no pejorative implications intended—Ed.) as to whether the statements made for the instrument were excessively enthusiastic (1). We are pleased to share some of the data upon which the statements were based.

Calcium and magnesium in serum were determined simultaneously, according to the method of Pybus et al. (2), except that no internal standard was used. With a Cordis Model 300 Autodiluter, 50 μl of each sample was diluted 100-fold in de-ionized water containing lanthanum chloride (1 g/liter) and dilute HCl (10 ml/liter). The Spectrophotometer was calibrated with a blank and three standards, each of which contained, per liter, 140 mmol of sodium and 5 mmol of potassium, and which were similarly diluted. Per liter, the standards contained 3.0, 6.0, and 7.5 mmol of calcium and 1.23, 2.46, and 3.09 mmol of magnesium, respectively.

Three different sets of samples were analyzed. One set of Reference Specimens was provided by the Clinical Chemistry Division, Manufacturer’s Assistance Program, at the Center for Disease Control, Atlanta. Another set, drawn from the Serum Control Pools at Hartford Hospital, Hartford, Conn., was supplied by G. N. Bowers, Jr. Table 1 gives comparative results for these two sets.

The third set, donated by S. Abercrombie and M. St. Cyr, consisted of 50 samples previously analyzed by atomic absorption at University Hospital, Boston. Once again, the two elements were determined simultaneously, according to the following procedure:

Each sample was analyzed five times in succession, with a four-second integration period. The instrument was then allowed to print out the mean value, SD, and CV. After every fourth sample, the blank solution was aspirated and the zero re-established by pressing “auto-zero”; then the calibration was refined by aspirating the appropriate standard and pressing “auto-cal.” We did not attempt to determine whether this frequent recalibration was in fact necessary.

Even with this unusually elaborate analytical procedure, all 50 samples were analyzed, after dilution, in 30 min, giving a rate of 36 s/sample or 18 s/element. The data appear in Table 2.

In a recent study of the determination of calcium in serum, Copeland et al. concluded that the use of a strontium internal standard would provide the best analytical accuracy (3). Because we judged that the agreement with reference samples shown in Table 1 was adequate, we did not pursue the internal-standard approach, although the instrument we were using could have readily permitted us to do so.

References


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<th>Ca, mmol/liter</th>
<th>Mg, mmol/liter</th>
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Table 1. Simultaneous Determination of Ca and Mg in Nine Serum Samples from Two Sources

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Mean bias (mean deviation):

- + 0.018 mmol of Ca/liter
- + 0.016 mmol of Mg/liter

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Ronald L. Stux
Robert H. Emmel
Herbert L. Kahn

Analytical Instrument Division
Instrumentation Laboratory, Inc.
Wilmington, Mass. 01887

For What Fluids is a Kit Useful?

To the Editor:

I urge that all kit manufacturers state whether the method is or is not applicable to blood, serum, plasma with different anticoagulants, urine, cerebrospinal fluid, or other fluids. Information provided with the kit or method desirably should include a statement that “there was no evaluation of the reliability of the procedures for fluids other than as indicated in the description of the method.”

Irwin Schoen
Automated Laboratory Services
Van Nuys, Calif. 91406

Urinary Cyclic AMP: Experience with Use of a Kit

To the Editor:

We have used Cyclic AMP Assay kit TRK 432 (Amersham/Searle Corp., Arlington Heights, Ill. 60005) to measure cyclic AMP in urine.

We collected 24-h urines in plastic bottles containing 300 mg of thymol as preservative. The volume of each collection was measured, one portion was set aside in the refrigerator for urinary creatinine measurement, and another portion stored at -15 °C for cyclic AMP analysis. Before analysis the frozen urine samples were thawed, centrifuged at 1000 rpm for 10 min, and diluted, usually 50- or 100-fold, with buffer, so that the concentration preferably fell within the range 0.5 to 4 pmol of cyclic AMP per assay tube.

The procedure described in the booklet supplied with the kit was followed, except for the following modifications:

De-ionized water [Barnstead No D0809 Ultrapure (mixed bed) cartridge] was used in lieu of distilled water. Freeze-dried reagents were dissolved in water and the solutions divided into two or four aliquots. These and the appropriately diluted standards (1, 2, 4, and 8 pmol of cyclic AMP per tube) were stored in disposable plastic tubes (No 2001, fitted with snap caps, 17 × 100 mm; Falcon, Division of Becton-Dickinson Co., Oxnard, Calif.) at -15 °C. A rack containing 34 12 × 75 mm conical polystyrene centrifuge tubes (Amersham/Searle No 196270) per batch was immersed in an ice-water bath. Instead of a round-bottom centrifuge tube we used conical-tipped ones because they are more efficient in packing light sediment by centrifugation. This is particularly true when the sediment is charcoal and a clear supernatant fluid is required for transfer. All aliquots of specimens and controls were measured in duplicate; standards were measured in triplicate.

After radioactive cyclic AMP was added to the standards and specimens, the tubes were centrifuged at 1500 rpm for about 10 s and then returned to the ice-bath before the binding protein was added. After the contents were vortexed for 15 s, the tubes were centrifuged once more at 1500 rpm for 10 s. The centrifugation forced any fluid adhering to the sides of the tubes down to the conical tips, thereby preventing loss and facilitating mixing with the reagents. After the charcoal suspension was added, the tubes were centrifuged in a RC-3 refrigerated centrifuge (Ivan Sorvall Inc., Norwalk, Conn. 06856) at 3000 rpm for 8 min.

The scintillating fluid used was Amersham/Searle ACS Aqueous Counting Scintillant No. 196290. Samples were transferred to appropriate vials containing the scintillating fluid, well mixed, and the radioactivity for each vial was counted in a UNILUX LSC (Searle Analytic Inc., Des Plaines, Ill. 60018) for 4 min (counting efficiency 30%).

A calibration curve $B_0/B$ vs. pmol of cyclic AMP per tube was drawn, where $B_0$ and $B$ are the counts per minute in the absence and in the presence of non-radioabeled cyclic AMP, respectively. The amount of cyclic AMP in each tube, in picomoles, was read off the linear graph and the concentration finally expressed as both umol/24 h and as umol of cyclic AMP per gram of creatinine. The precision of the micropipettes was checked each month. Urine controls consisted of 100-ml quantities of 10 pooled urines, divided into 2 ml aliquots and stored in plastic 17 × 100 mm capped tubes at -15 °C.

With the technique described and use of this kit, we found:

Precision: Within-assay: $n = 20$ specimens in duplicate, mid-normal range: CV = 5%. Between-assay: $n = 13$ measurements on aliquots of a pool, mid-normal range: CV = 6.4%.

Analytical recovery: $n = 13$ different additions, $X = 98\% (50-105\%$).

Normal ranges: We tested 42 healthy subjects without history of bone, renal, or endocrine disease, 24 men 25 to 58 years old and 18 women 25 to 68 years old. A highly significant sex-related difference ($P < 0.001$) was found when results were expressed as micromoles of cyclic AMP per gram of creatinine: for men, 1.39 to 4.13, for women, 2.56 to 5.28. Expressed as micromoles of cyclic AMP/24 h, there was no such significant difference ($P > 0.5$). The normal range for all 42 of the subjects was 2.35 to 6.49 pmol/24 h. Distributions were gaussian for both men and women, whichever way the results were expressed. Consequently the above ranges are based on means ± 2 SD. These are compared with some values previously published in the literature (Table 1).

Table 1. Reported Normal Ranges for Urinary Cyclic AMP

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* Values converted to mean ± 2 SD from the published data.

References