those of hypocalcemia, so assays for both magnesium and calcium are often requested on the same specimen. In our department, about half of all requests for magnesium over the past 4½ months have also been for calcium.

A rapid kit procedure for estimating calcium (the "60-second" calcium kit; American Monitor International, Kennedy Way, Belfast, N. Ireland) was recently evaluated (1).

American Monitor International has also supplied a "60 second" kit for assay of magnesium, the method being based on formation of a complex between magnesium and the metallochromic dye, Calmagite, and measurement of the resulting absorbance (2).

This kit has not yet been evaluated in the literature. Here, I present data on the precision, accuracy, linearity and stability of the method.

The kit contains two reagents, each in duplicate. One is the dye reagent, which contains Calmagite (0.2 mmol/L), EGTA [ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid] to eliminate calcium interference by preferential chelation of calcium, and dimethyl sulfoxide to minimize the effects of lipemia. The other is a pH 11.6 buffer, which also contains nonreactive surfactants to minimize the effects of lipemia and a complexing agent to prevent interference from heavy metals. Both reagents come in 100-mL plastic bottles with a total volume of 400 mL per kit.

The kit also includes a 5-mL aqueous magnesium standard (1 mmol/L), which contains a nonreactive preservative. Reagents and standard may be stored at room temperature. The working buffered-dye reagent is prepared by mixing equal volumes of dye and buffer. (Some frothing may occur on mixing, so it is advisable to prepare slightly more reagent than is needed.)

Fifty microliters of standard, quality control, serum, or de-ionized water (for the blank) is added to 5 mL of working reagent. After mixing, the solutions are left to stand for 1 min before the absorbance is measured at 547 nm against the blank.

I assessed precision of two concentrations, using "Hyland Q Pak One and Two" human-based control sera (Travenol Laboratories, Lessines, Belgium) (Table 1).

I evaluated accuracy by comparing the results for 33 patients' sera as obtained by using the kit (γ) and a routine atomic absorption spectrophotometric procedure (x). A correlation coefficient of 0.93 was obtained. The equation for the line of best fit was y = 1.03x - 0.04.

Values obtained with the kit gave an average negative bias of 19 μmol/L over atomic absorption spectrometry.

Linearity, assessed by assay of serial dilutions of a 10 mmol/L stock aqueous solution of magnesium sulfate, extends to approximately 2.8 mmol/L. The final color produced by the reaction is stable for up to an hour after the start of the reaction.

In summary, the kit performed well, and the claims of the manufacturer regarding stability and linearity were substantiated. The broad linearity range is a clear advantage. The kit shares many similar features to that of the "60-second" calcium kit previously evaluated (1). In both methods 50 μL of sample is used, but the manufacturer claims that as little as 20 μL may be used, and in both cases equal volumes of buffer and dye are prepared. However, two advantages of the magnesium kit are that both reagents may be stored at room temperature, whereas the dye reagent in the calcium method is stored at 4 °C, and the working buffered-dye reagent in the magnesium method is claimed to have a greater stability, three months as opposed to only 4 h for the calcium kit.

The number of calcium requests received each day by a clinical biochemistry laboratory probably limits the use of the calcium "60-second" method to an out-of-hours procedure. If the number of magnesium requests is substantially less (in our laboratory, 84 requests have been received over the past 4½ months) the kit may appropriately be used as a routine procedure.

It is both simpler and safer than atomic absorption spectrometry, so that even the most junior member of staff may use this kit with confidence. In addition, it requires a smaller sample volume and results are obtained more quickly. It should provide a useful adjunct to the "60-second" calcium kit.

Table 1. Within- and Between-Batch Precision Data for the "60-Second" Magnesium Kit

<table>
<thead>
<tr>
<th></th>
<th>Within-batch</th>
<th>Between-batch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Q Pak One</td>
<td>0.70</td>
<td>0.020</td>
</tr>
<tr>
<td>Q Pak Two</td>
<td>1.67</td>
<td>0.033</td>
</tr>
<tr>
<td>n = 20 each.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

To the Editor:

Peptide hormones in biological samples are generally measured by radioimmunoassays (RIAs) that involve specific antibodies raised in rabbits. In some assays a second antibody, directed against rabbit IgG, is used to separate bound and free hormone: the second antibody either is coupled to an insoluble support or is added in solution with carrier IgG to create a visible precipitate. Because more and more commercial RIA kits are now based on the second-antibody solid-phase principle, many patients' samples will be so measured.

We have encountered several patients (12 in the last six months) with falsely increased results for RIAs involving the second-antibody solid-phase system. The interference was caused by antibodies to rabbit IgG in the sera of these patients, all of whom had been in intimate contact with rabbits as pets. When RIAs with a different separation system were used, results for these patients were within normal limits.

We evaluated the following RIA methods:

A. With double-antibody solid-phase separation: 1. Lutropin commercial kit, 125-I-hLH "Coatria" (BioMérieux, Charbonnières-les-Bains, France). 2. Folitropin commercial kit, 125-I-hFSH "Coatria" (BioMérieux). 3. Thyrotropin in-house method. First antibody and hormone to be labeled were obtained from Calbiochem Behring, San Diego, CA; the second antibody, on solid phase, was from Organon, Oss, The Netherlands; the standard was the WHO 1974 1st IRP. 4. Prolactin commercial kit, PROL-RIA (IRE, Fleurus, Belgium). 5. Estradiol commercial kit, 125-I-Oestradiol radioimmunoassay kit (EIR, Würlingen, Switzerland).

B. With other separation procedures: 1. Folitropin in-house method. First antibody was obtained from UCB, Brussels, Belgium; human folliculin for labeling from Kabi AB, Stockholm, Sweden; standard who 1974 1st IRP. 2.
Lutropin in-house method. First antibody and hormone to be labeled were purchased from Kabi AB; standard who 1974 1st IRP. 3. Thyrotropin, same as A-3 except we changed the bound/free separation step. For assays B-1 through B-3, bound hormone was separated from the 1-mL incubation mixtures by adding 100 µL of diluted (25 mL/L) normal rabbit serum and 100 µL of diluted (125 mL/L) second antibody (both obtained from Antibodies Inc., Davis, CA), and 100 µL of a 300 g/L polyethylene glycol solution. After incubating the mixture for 30 min at 4 °C, we collected the precipitate by centrifugation (1500 × g, 5 min, 4 °C). 4. Prolactin commercial kit, PROLK-PR (CIS, St. Quentin-Yvelines, France). 5. Estradiol in-house method. Bound and free hormone were extracted and separated with dextran-coated charcoal as published earlier (1).

Binding of normal rabbit IgG by patients’ sera: We labeled 2 µg of IgG, prepared by precipitation with ammonium sulfate from normal rabbit serum (Antibodies Inc.), with Na125I (Amersham International, Amersham, U.K.) with lactoperoxidase as previously described (2). We incubated 50 pg of labeled rabbit IgG overnight at 4 °C with 100 µL of patients’ serum, with and without addition of 5 µg of normal rabbit IgG, added 100 µL of the polyethylene glycol solution, continued the incubation for 30 min, and collected the precipitated complex by centrifugation at 2000 × g for 30 min. Results were expressed as the percentage of the added amount of tracer that was bound.

Table 1 summarizes our results for sera from four patients. All values in the group A assays are grossly increased. Because the results were not compatible with the clinical picture according to the attending physician, we suspected an artifact in the assays. As a first screening test, we assessed whether there was parallelism between the unknown samples and the standard curves of the different assays. Results from patients with increased values that agreed with their clinical status did show parallelism, but the results for the patients listed in Table 1 decreased disproportionately. This indicates that the serum of the four patients contained an interfering substance. We then reassayed these patients’ sera by techniques involving a different principle for the bound/free separation (Table 1, group B) and obtained values that were consistent with the clinical picture.

We considered the most probable cause of interference to be an antibody in the patients’ serum directed against rabbit IgG. Upon questioning, we learned that all patients had one or more rabbits as pets. When we incubated samples of these four patients’ sera with labeled rabbit IgG, all bound the label significantly more than did normal sera. Moreover, we could displace the bound labeled IgG with unlabeled rabbit IgG, thereby confirming the presence of an anti-rabbit IgG antibody in the sera of these patients. This antibody will compete with the second antibody on the solid phase for the labeled hormone–first-antibody complex, thereby lowering the precipitated amount of radioactivity. The interference can be overcome by adding rabbit non-immune IgG in the first incubation step and appropriately more second antibody (not supplied with commercial kits).

Systems in which polyethylene glycol is used in precipitating the antibody complexes are not affected.

The same type of antibody interfering with the thyrotropin assay has been described after vaccination with a vaccine containing rabbit IgG (3). A human antibody to rabbit IgG was responsible for spurious increases of thyrotropin values in an IRMA, although the underlying mechanism was different (4). These authors state an incidence rate of 5 to 20% in persons employed in animal-care facilities.

In view of this incidence rate we thus advise abandoning the use of double-antibody solid-phase separation systems in RIA procedures, especially because adequate alternative procedures are available. This can save patients from further expensive, unpleasant, and unnecessary investigations, initiated on the basis of falsely increased results of hormone determinations.

We thank the WHO International Laboratory for Biological Standards, Holly Hill, Hampstead, London, for providing the WHO standards; Dr. F. H. de Jong for performing the estradiol assays; Miss G. Boe for skillful technical assistance; and Miss M. Synderhoud for expert secretarial help.

References

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Serum Creatine Kinase: Relationship to Lean Body Mass in a “Real-Life” Situation

To the Editor:

Athletes have high baseline values for serum creatine kinase (CK, EC 2.7.3.2) (1, 2). This suggests a relationship between (muscle-derived) CK concentration in serum and muscle mass. Indeed, such a relationship has been demonstrated (3) in a group of fit men who abstained from physical activity for 48 h before blood was sampled. This study showed a good correlation between serum CK and lean body mass, expressed in percentage of body weight. We had the opportunity to examine the practical significance of this relationship in conditions where the physical activity of the subjects cannot be controlled.

The study group consisted of male shiftworkers in a steel plant. The shift schedule involved 21 consecutive days of work followed by seven days off. Of

| Table 1. Relation between Separation System Used and RIA Hormone Results in Four Patients |
|-----------------------------------------|---------|---------|---------|
| Assay                                  | Group A |         |         |
|                                        | MH      | VE      | AK      | DM      |
| Lutropin, int. units/L                 | 135     | >200    | 105     | 140     |
| Prolactin, int. units/L                | 37      | 73      | 20      | 48      |
| Thyrotropin, int.-int. units/L         | 22      | —       | 25      | 0.8     |
| Estradiol, nmol/L                      | 3.1     | 5.8     | 0.8     | 0.2     |

Group A assays: double-antibody solid-phase systems to separate bound and free hormone; Group B assays: other techniques (see text). Results are means of duplicate determinations on a typical sample from each patient. Similar results were obtained for at least five different sera from each patient.