interferent error is the rate constant of the interfering substance (4). Because this rate constant is temperature dependent, the temperature differences occurring in the macro and micro creatinine methods may give rise to dissimilar acetocacetate rate constants, causing the former to show large negative ketone interference while the latter shows only slight interference.

Ketoadiosis observed in patients can reach values of 10 mmol/L. Its effect on overestimation of creatinine values in some methods has been a cause of concern. We report underestimation of creatinine values with the macro KDA method, an effect that may have more serious clinical implications than overestimation because kidney impairment may be missed and, in general, clinicians are less likely to question normal results.

We advise caution in interpreting KDA creatinine values in specimens containing ketones. Parameter changes on the macro KDA system are being initiated by American Monitor to correct this problem.

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On Standards Provided with Digoxin Radioimmunoassay Kits

To the Editor:

Discrepancies between results with various digoxin RIA kits are partly ascribable to standardization problems (1–3). To study these, we obtained standards from each of four commercial suppliers: Becton-Dickinson Immuno-diagnostics, Orangeburg, NY (a); Pharmacia Diagnostics AB, Uppsala, Sweden (b); Clinical Assays Inc., Cambridge, MA (c); and New England Nuclear, North Billerica, MA (d). Each standard, in concentrations of 1, 2, and 4 μg/L, was assayed eight times during 38 days of refrigerated storage as recommended by the kit manuals. Assays were performed both directly and on extracts. Our own standards in plasma, used for comparison quantification, were prepared and stored as described elsewhere (4) and were kept frozen in portions until used. We added 800 μL of isotonic saline to 200 μL of the samples, then extracted them with 5.0 mL of dichloromethane. After centrifugation at 4 °C, a 4.0-mL sample of the extract was evaporated and the residue was dissolved in 150 μL of heparinized plasma. Extracts of plasma were then examined each assay day in duplicate, and a parallel assay series was run on non-extracted samples. Reagents from kit b were used in all these assays. We evaluated results as described elsewhere (5). Student's t-test of paired comparisons (p < 0.05) was used to evaluate differences.

Results for kit a are given in Figure 1. In the text, figures are given for the 2.0 μg/L concentration, which is the suggested upper therapeutic limit. When the standards were assayed directly, several significant differences were found among the four commercial standard preparations. Maximum differences were 0.31 μg/L at 1.0 μg/L, 0.56 μg/L at 2.0 μg/L, and 0.53 μg/L at 4.0 μg/L. Significant differences were also found between the commercial standards and the expected values (our own standards). Kits b and d underestimated at 1.0 μg/L, kits a (1.91 μg/L; p < 0.05), c (1.83 μg/L; p < 0.01), and d (1.55 μg/L; p < 0.001) at 2.0 μg/L, and all kits at 4.0 μg/L.

The digoxin concentrations found after extraction exceeded those assayed directly during the first 10 days (the differences were significant for all concentrations with kits c and d and at 1.0 μg/L for kit b). The amount accounted for after extraction was subsequently lower for all kits, especially for kit a (Figure 1) and b (about 30%). The maximum differences between kits (first 10 days) were 0.32 μg/L for the 1.0 μg/L standard, 0.87 μg/L for the 2.0 μg/L, and 0.76 μg/L for the 4.0 μg/L. Significant differences were found between kits. Kits c and d gave significantly higher values than anticipated for the 1.0 and 2.0 μg/L standards (2.28 μg/L, p < 0.01, and 2.22 μg/L, p < 0.001, respectively) and kits b and c significantly lower values for the 4.0 μg/L standard.

We have shown that our preparation of reproducible standards presents no

1 Figures and tables giving results for all kits are available from the authors on request.

Fig. 1. Measured digoxin concentration in commercial standard preparation at concentrations of 1, 2, and 4 μg/L for kit a

Mean values and 95% confidence limits are given. ——— RIA without extraction; ——— RIA of extracts. Numbers at right emphasize intended concentrations.
difficulties (6). The differences found may have several other explanations, such as use of impure digoxin in standards. Furthermore, the commercial standards were prepared in a matrix of human serum but, like our routine samples, our standards were in heparinized plasma. The kit manuals do not suggest any effect of this difference. In an earlier study (6) we found that the standard curve (bound cpm vs concentration) for standards prepared in heparinized plasma was steeper than it was when serum was used. In commercial kits for cortisol assay the native matrix used as standard medium in the kits was altered by the preparation procedure employed, which affected accuracy (7). This may be important also for digoxin RIAs.

The low analytical recovery of digoxin in extracts of standards after the first 20 days of storage was unexpected. Free fatty acids increase during storage of serum, even at low temperatures (8). Digoxin and other steroids can be entrapped in micelles of free fatty acids, which may disturb direct RIA (9, 10) and also possibly decrease extraction into dichloromethane. Bacterial contamination of the standard preparations may influence digoxin RIA (11), but we detected no bacterial growth in the affected standards.

Reliable standards are a prerequisite for useful monitoring of digitalized patients, as is also the use of the same therapeutic range in different hospitals. Our results indicate a need for better control by the kit supplier. We suggest that digoxin be calibrated against the internationally approved substance fulfilling the specifications in the international pharmacopoeia and urge the use of specified standard media resembling as closely as possible the samples collected. Standards should not be used after refrigerated storage for longer than one week.

References

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EDTA in Vacutainer Tubes Can Interfere with Plasma Amino Acid Analysis

To the Editor: Screening plasma and urine for amino acid abnormalities is an essential part of the biochemical investigation of patients suspected of having an inborn error of metabolism. Recently, we were involved in the investigation of a child; ion-exchange chromatography of the plasma amino acids showed an unusually increased ninhydrin-positive peak, which eluted like authentic methionine sulfoxide with our citric acid–sodium citrate buffer program (1), but with a different 570/440 nm ratio (methionine sulfoxide 5.4, unknown peak 4.7). The pattern for urinary free amino acids showed no abnormalities, and the patient was receiving no medication. On two different days, plasma amino acids of blood samples collected in sterile Vacutainer Tubes (Becton Dickinson) containing tripotassium EDTA solution demonstrated the same abnormality. The possibility of an interfering substance present in this type of tube was then tested as follows. We added 1 mL of distilled water to each of six such Vacutainer Tubes of different capacity (3-, 5-, and 10-mL), acidified with 50 mg of sulfosalicylic acid, centrifuged, and then chromatographed 50 μL of the supernate. Each sample gave the unknown peak. Chromatography of aqueous saturated solutions of tripotassium or disodium EDTA did not demonstrate any ninhydrin-positive compounds. Some α-amino products of the thermal decomposition of EDTA and its salts in aqueous solutions were previously described (2), and one of them was also produced during sterilization by gamma-ray irradiation of Vacutainer Tubes containing tripotassium EDTA in aqueous solution. Probably this product is present in all the manufacturer’s sterile tubes that contain EDTA salts in aqueous solution.

References

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Analytical Differences in Measurement of Plasma Catecholamines

To the Editor: Measurement of catecholamines in human plasma offers a considerable analytical challenge. In recent years this has been met by an almost exponential growth in publications, most of them representing modifications to the liquid chromatography with electrochemical detection (LCEC) approach (1, 2) or to the radioenzymatic assays (REA) (3, 4). Despite the extensive literature, there have been surprisingly few comparisons between results by these entirely different analytical procedures (2, 5). There is a noticeable variation in catecholamine results re-