blanks. Interestingly, the spectra of these samples were also improved: background noise was decreased. We confirmed this finding with many other samples. The organic layer was cleared by centrifugation and the fluorescence signal was easy to measure (Figure 1c).

This modification allows an accurate determination of MDA with strongly hyperlipemic samples. We recommend that this treatment be included in all MDA assays of clear or turbid serum samples.

References

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Nonlinearity of Calcium Measurement on the Kodak Ektachem at Concentrations > 110 g/L (Slide Generations 18 and 19)

To the Editor:
The Ektachem (Eastman Kodak, Rochester, NY) slide for measuring serum calcium is based on a change in absorption when calcium binds the dye Arsenazo III. The manufacturer has claimed an analytical range of 10–160 mg/L for the Ektachem Ca slide. During routine patient comparisons, we noticed that patients' samples with Ca values >120 mg/L consistently produced lower Ca values with Generations 18 and 19 Ektachem Ca slides than with another automated method in our laboratory that utilizes the Ca-binding dye cresolphthalein. For 10 samples having Ca values of 130–160 mg/L by the cresolphthalein method, we found the Ektachem values to average 15 mg/L lower (maximum, 25 mg/L lower). Samples with Ca values <110 mg/L had excellent agreement on the two instruments (± 3 mg/L). We therefore performed linearity studies with both methods to examine these discrepancies. Parallel dilutions of plasma samples with Ca values >170 mg/L were prepared with use of the manufacturer's bovine albumin-based diluent, which we further diluted with a half-volume of water to reduce the Ca content of the diluent. The diluent as used had a Ca concentration of 22 mg/L and a total protein content of 660 g/L as measured by the Ektachem.

As seen in Figure 1, all samples with Ca values <105–110 mg/L produced values within 3 mg/L of their expected value by both methods. In addition, both methods had a linear response for samples with Ca concentrations <110 mg/L, indicating that the diluent was not producing a "matrix effect" that could complicate interpretation of the study. However, for samples with Ca values >110 mg/L, there was an increasing disparity between the Ektachem and the other method as Ca concentrations increased. For instance, the Ektachem produced values that were 7–14 mg/L lower than expected at Ca concentrations of 120–130 mg/L and 20 mg/L lower at Ca concentrations of 150–160 mg/L. On the basis of these data, we informed the manufacturer and began repeating all Ca determinations performed on the Ektachem, using a 1:2 dilution if the original value was >105 mg/L.

We have become accustomed to minor changes in patients' values (2–3 mg/L) between different generations of Ektachem Ca slides, but these data demonstrate that Generations 18 and 19 Ektachem Ca slides have a clinically significant nonlinear response above 110 mg/L. The manufacturer recently provided customers with a software update and new Supplementary Assigned Values for Ca calibrations, which has improved the linear response to 140 mg/L in our laboratory. Because this problem existed for at least several months before the software update, it is important that users be aware that reported Ca values from hypercalcemic patients determined with Generations 18 and 19 Ektachem Ca slides had the potential to be falsely low at concentrations beyond 110 mg/L.

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Editor's note: The manufacturer's representative offered no additional reply for publication.

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Digoxin Immunoassay and Chinese Medicine

To the Editor:
We read with great interest the Letter on Chinese medicine and digoxin assay reported by Pansar (1). Unfortunately, however, our previous reports (2,3) on this problem were not cited. We had clarified that Chinese medicine containing Ch'an Su had immunoreactive digoxin-like activity.
We analyzed eight components of Chinese medicine and only Ch'an Su (Kyushin Pharmaceutical Co., Tokyo, Japan) had strong immunoreactivity, but weak reaction was also observed in the Zhu Dan component (Kyushin Pharmaceutical Co.). Digoxin-like immunoreactivity of Ch'an Su solution (10 mg/L) showed 3.68 μg/L by the Abbott TDx analyzer, 4.35 μg/L by Du Pont 6800 V discrete analyzer, and >5.0 μg/L (off-range) by the Enzymun-Test kit. These different values were attributed to the different cross-reactivity of antibodies to bufalin, bufotalin, and (or) cinobufagin in each method (3).

Volunteers took two tablets of Kyushin (one tablet contained 0.83 mg of Ch'an Su) three times a day, a common daily dose of this drug. In their sera, digoxin-like immunoreactivity appeared 1 h after drug intake and reached almost 0.3–0.4 μg/L within 12 h in all three methods. Cardiotoxic steroids in Ch'an Su may have weak biological activity but strong immune cross-reactivities. Therefore, patients taking both digoxin and Chinese medicine may not become actually toxic, even though they have a high, supposedly toxic value for digoxin-like immunoreactivity.

Chinese medicines are easily obtainable at common pharmacies without a doctor's prescription in Asian countries; in Japan, >300 kinds of tablets or powder contain Ch'an Su. Therapeutic monitoring of digoxin should be performed after cessation of intake of Chinese medicine.

References

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The author of the Letter replies:

To the Editor:

I take Fushimi and Amino's point that Ch'an Su, which contains bufadienolides, may cause problems with digoxin immunoassays; however, as I pointed out in my Letter (1), not all the digoxin assay kits I tested gave an apparent digoxin concentration after the oral intake of Lu-Shen Wan pills containing Ch'an Su. I am surprised that, in view of the 48-fold higher apparent digoxin (by Enzymun-Test) in a Ch'an Su solution as reported by Fushimi et al. (2, 3), the measured plasma concentrations of digoxin are the same by all three assays.

Ch'an Su consists of the dried secrections from the postauricular and skin glands of the toad Bufo bufo gargarizans. The resin-like secretions contain several different bufadienolides, e.g., bufalin, cinobufagin, cinobufotalin, and resibufogenin; to my knowledge the exact components, their concentrations, and their cross-reactivities with digoxin antibodies are not known. Even amongst Lu-Shen Wan pills, there are regional preparations of the pill in China with variable contents of bufalin, cinobufagin, and resibufogenin (4). Until these Chinese medicines containing Ch'an Su are fully characterized for their bufadienolide content, one must be wary of cross-reaction problems with some of the current immunoassays for digoxin.

In the Far East the usage of herbal medicines is quite common, and preparations containing Ch'an Su are used for various ailments, including cardiac problems, as Fushimi et al. (2, 3) have stated. Rather than suggesting that "these different values [are] attributed to the different cross-reactivity of [digoxin] antibodies to bufalin, bufotalin, and (or) cinobufagin in each method" and advocating that patients be taken off their Kyushin tablets before therapeutic drug monitoring for digoxin, attempts should be made to identify the exact bufadienolides in these preparations and to develop specific immunoassays for them, to facilitate therapeutic drug monitoring. Such attempts may also help alleviate the phenomenon of "digoxin-like immunoreactive substance.

References


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Mini- Gel PAGE for Enhanced Resolution of Polymerase Chain Reaction Detection of AFsoe Deletion in Cystic Fibrosis

To the Editor:

The predominant mutation (~70%) in cystic fibrosis (CF), an autosomal recessive disease, results from a base pair (bp) deletion (AFsoe) within the gene encoding the CF transmembrane conductance regulator (1). Genetic identification of CF by polymerase chain reaction (PCR) requires detection of amplified 98-bp (wild-type), 96-bp (homozygous CF), or 98/96-bp (heterozygous CF) products (2). This can be achieved by allele-specific 32p-labeled cDNAs on Southern and dot blots (2–4), solid-phase mini-sequencing (5), restriction endonuclease mapping (6), or generation of specific methylation sites (7). Although polycrylamide gel electrophoresis (PAGE) with ethidium bromide staining (8–10) provides a simpler alternative, we observed that the resolution of the 98-bp and 96-bp PCR products under traditional PAGE conditions (11) was poor, time consuming, and generally inadequate for the accurate and rapid genetic identification of suspected CF patients or carriers. In contrast, we found that by simply increasing the concentration of gel matrix cross-linker (bis-acrylamide) we could resolve the 98- and 96-bp PCR products on mini gels, thus shortening electrophoretic time and facilitating identifying the ΔF508 mutation.

Genomic DNA was obtained from dried neonatal bloodspots (3 mm diameter) by incubation for 1 h at 25 °C in 135 μL of 10 mmol/L Tris-Cl, pH 8.3, containing 50 mmol/L KCl, 2.5 mmol/L MgCl₂, 1 mmol/L Triton X-100, and 0.4 g/L gelatin, and then at 95 °C for 10 min with a mineral oil overlay (9). We then added a stock PCR reaction mix (67 μL) containing primers (2), a final concentration of 100 μmol/L of each dNTP (dATP, dUTP, dCTP, and dGTP), 2 mmol/L MgCl₂, 1 U of AmpliTaq DNA polymerase,