We believe that the erroneous values can be attributed to presence in the patients’ samples of a cross-reactant with the antibody to TSH used in the Tandem-E kit. The bead on which the TSH antibody is coated is washed before the addition of the substrate, which makes it unlikely that an interference with the indicator system is present. Regardless of the source of problem in this kit, we found that the Tandem-E gave erroneous results that were clinically significant for 10% (seven of 67 samples) of our adult male population.

Because of the potential for misdiagnosis or improper medication, our laboratory has discontinued the use of the Hybritech Tandem-E TSH reagent kit and is now using the Tandem-R HS kit.

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Stability of Individual Carotenoids, Retinol, and Tocopherol in Stored Plasma

To the Editor:

Craft et al. (1) recently examined the stability of individual carotenoids, retinol, and tocopherol in stored plasma. We would like to confirm their observations.

In the recent Nutrition Survey in Great Britain, jointly organized by the Department of Health, Ministry of Agriculture, Fisheries and Food; and the Office of Population, Census, and Surveys, U.K. (2), we regularly measured (3) these vitamers in a sample of date-expired, citrated transfusion plasma (Blood Transfusion Service; BTS) from October 1986 to September 1987. The collection of survey samples was organized in four two-monthly periods. We compared the means obtained in each of these four phases for four carotenoids—β-carotene, α-carotene, lycopene, and β-cryptoxanthin—and for retinol and tocopherol, to assess the stability over the year of the survey.

The BTS plasma sample was dispensed into 5-mL vials and was stored at −20 °C. Vials were further dispensed into 0.5-mL tubes when required, the contents of a tube being thawed and used only once. Each sample of plasma analyzed was therefore thawed only three times while in our hands. The samples were not flushed with nitrogen or fortified with ascorbic acid, but butylated hydroxytoluene was added to the extracting solvents (3).

The BTS sample was the first sample analyzed (3) with each batch of survey samples and was used as a quick check on daily performance. We made 102 measurements over the 12 months. The results (Table 1) indicate that mean retinol and tocopherol concentrations were not significantly different in the four phases. In contrast, mean concentrations of β-carotene, β-cryptoxanthin, and lycopene differed significantly among the four phases and showed a significant downward trend over the course of the survey. Mean α-carotene concentrations were not significantly different among the four phases. The concentration of α-carotene was the lowest of those measured, and precision was poorest for this analyte (3); hence this may be the reason why no changes in concentrations were detected.

Except for retinol, the SD values for the nutrients were higher in phase I than at any other time in the survey. This possibly indicates that precision was poorer at that time because the method had been set up only recently. However, it does not interfere with the interpretation of the results because mean values decreased mainly after phase II. The data suggest that carotenoid concentrations in plasma decrease after six months of storage at −20 °C, but such plasma is suitable for use in the quality control of retinol and tocopherol for at least 12 months.

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References

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Lactate dehydrogenase in Lung Cancer

To the Editor:

In the report on lactate dehydrogenase (LDH, EC 1.1.1.27) and its isoenzymes by Rotenberg et al. (1), it appears that the investigation of LDH isoenzymes was done on samples that had been stored at 4 °C to 8 °C for up to 24 h. In standard clinical biochemistry textbooks one finds that some authors do not specify the effect of temperature on the isoenzymes (2), while others do state that there is a variable decline in total LDH activity if the sample is refrigerated for three or four days. Also, heat stability of some isozyme fractions could be exploited to investigate the changes in isozyme concentrations by incubating samples at 60 °C, at which point LDH-4 and LDH-5 are destroyed (3).

Table 1. Concentrations of Fat-Soluble Vitamins in Quality-Control Plasma Measured in the Four Phases of the British Nutrition Survey, October 1986 to September 1987

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Phase I (n = 24)</th>
<th>Phase II (n = 24)</th>
<th>Phase III (n = 28)</th>
<th>Phase IV (n = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinol</td>
<td>2.08 (0.306)</td>
<td>2.23 (0.396)</td>
<td>2.16 (0.360)</td>
<td>2.04 (0.188)</td>
</tr>
<tr>
<td>Tocopherol</td>
<td>23.22 (3.244)</td>
<td>24.80 (1.204)</td>
<td>23.29 (2.699)</td>
<td>23.58 (1.904)</td>
</tr>
<tr>
<td>β-Cryptoxanthin**</td>
<td>0.305 (0.12)</td>
<td>0.295 (0.045)</td>
<td>0.292* (0.039)</td>
<td>0.225* (0.031)</td>
</tr>
<tr>
<td>Lycopene*</td>
<td>0.825 (0.344)</td>
<td>0.710 (0.088)</td>
<td>0.688* (0.156)</td>
<td>0.854* (0.137)</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>0.066 (0.022)</td>
<td>0.067 (0.009)</td>
<td>0.067 (0.016)</td>
<td>0.061 (0.015)</td>
</tr>
<tr>
<td>β-Carotene**</td>
<td>0.152 (0.043)</td>
<td>0.145 (0.016)</td>
<td>0.141* (0.020)</td>
<td>0.118* (0.014)</td>
</tr>
</tbody>
</table>

ANOVA: *P < 0.02. **P < 0.001. Different suffixes indicate differences between means: P < 0.05 & P < 0.1 by the Schefe test. Values are means (and SDs), μmol/L.
However, Moss et al. (4) offer us good advice, i.e., to analyze isoenzymes one should store serum samples at room temperature, because LDH-4 and LDH-5 are labile in the cold and show a significant loss of activity. This was also confirmed in the review article by Lott and Stang (5). Such a loss is, however, prevented by addition of NAD+ (100 mg/L) or glutathione (31 mg/L), which decreases the rate of inactivation. Possibly Rotenberg et al. stabilized the isoenzymes by some modification or observed no decrease in activity at 4°C but found it inappropriate to report the details in their article. If this is not the case, two possible conclusions can be drawn from their observations: first, the sensitivity of the isoenzyme data (LDH-4 and LDH-5 fractions) was falsely suppressed because of the reduction of the activity of these isoenzymes; second, because of the reduction of LDH-4 and LDH-5 fractions the proportion of the total LDH activity formed by the remaining of the isoenzymes may have been falsely increased.

It has been stated that not all investigators are in agreement on the effect of cold on LDH-4 and LDH-5 fractions (4). I therefore feel that it is important that this aspect should be clarified, especially if the test is to be considered as another tool to predict response to therapy and prognosis, not only in lung cancer but in many other clinical conditions.

References

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Parasquat Measured in Serum with the Abbott TDx

To the Editor:

Parasquat, a bipyridinium herbicide, has been implicated in several fatalities (1). Its measurement in serum can help predict the probable outcome of poisoning and aid in clinical management (2). Techniques developed to measure parasquat in biological fluids (3) include a simple, rapid fluorimunoassay developed in our department (4). A rapid assay adapted for use on readily available instrumentation is the ideal. The Abbott TDx is used in many laboratories, but no assay for parasquat in the TDx is currently available. However, existing software and assay protocols can be utilized, and we describe the adaptation of the Digoxin II assay procedure for measurement of parasquat.

The preparation of antiserum and fluorescent label, described previously (4), only required pre-dilution to prepare stock solutions acceptable to the TDx. The label was diluted in TDx phosphate buffer (53 μL in 24 mL) to give a concentration of 160 nmol/L. On dilution in the instrument this gave a final concentration of 2 nmol/L. The antiserum titer, having been assessed previously (4), required a 75-fold pre-dilution to give a final 6000-fold dilution in the instrument. Spare TDx reagent containers were washed thoroughly and re-filled as follows. Container P, fresh buffer; container S, antiserum pre-diluted 75-fold; container T, label pre-diluted 53 μL/24 mL. These replacement containers were placed in a Digoxin II container for use and the bar-code reader accepted the reagent pack as a Digoxin II assay. Standards and samples were pre-treated by protein precipitation as described in the Digoxin II assay, 300 μL serum being mixed with 300 μL of pre-treatment reagent and centrifuged before use. The only assay variables that had to be changed were the individual standards concentrations.

For a standard curve to be accepted and stored by the TDx, the polarization decrease between standards and the maximum and minimum polarization readings must meet certain criteria. We did a preliminary calibration run, using parasquat standards (in human serum) of 0, 50, 200, 500, 1000, and 2000 μg/L concentration and plotted the results on semi-log graph paper. From this it could be seen that, whereas the maximum and minimum mP readings of the instrument were acceptable, the standards did not comply with the required range of mP variations. The instrumental requirements for individual mP variation was satisfied by using standards of 0, 25, 60, 150, 400, and 1800 μg/L concentration. Under these conditions the TDx accepted the standards for assay (Figure 1).

The sensitivity of the assay was calculated from data on 20 replicates of the zero standard (5). The minimum detectable concentration was 3 μg/L within 95% confidence limits. We assayed 35 normal sera and found them to have a mean value not significantly different from the zero standard, the polarization reading having an SD of 2.26 and a CV of 1.21%. An alternative and perhaps more relevant minimum detectable concentration, 5 μg/L, was calculated from the polarization reading 3 SD from the mean value for these 35 normal sera.

To evaluate precision we used human serum with weighed-in amounts of parasquat. The calculated within-assay CV of 10 replicates was 4.8% at 75, 3.2% at 165, and 6.1% at 300 μg/L.

We assayed 19 sera from the Metabolism and Pharmacokinetics Section, Central Toxicology Laboratory, ICL, Macclesfield, Cheshire, U.K. containing parasquat in concentrations ranging from 72 to 1640 μg/L, and the measured values correlated well (r = 0.96) with their reported values.

A simple assay for serum parasquat is needed (3). Until a commercial assay is available, the adaptation of existing software and protocols from equipment such as the TDx would appear to be a satisfactory alternative.

References