tests are now available to the laboratory.

Newer methods that may show promise, although I have no experience with them, include the method of Price et al. (7), enzyme immunoassay (8), and the method of Swanson and Walters (9). Also available is the fluorescent polarization immunoassay procedure used in the TDx analyzer; I have evaluated this method and found the results correlate fairly well with those by liquid chromatography. Thus, this method provides a rapid, reliable method for acetyaminophen quantification, although it requires somewhat expensive equipment and reagents.

An inexpensive and rapid colorimetric procedure introduced by Liu and Oka in 1980 (10) is the basis for a commercial kit now available (cat. no. 05806; Stainbo Laboratories, Inc., San Antonio, TX 78202). A version of this method has been used in my laboratory since 1981, at which time we required a screen for acetyaminophen to give our physicians an idea of how to manage the patient until a quantitative result could be obtained. I modified this method so that it could be used as a 5-min qualitative screening test, with verification of the results to follow with our slower but more accurate quantitative liquid-chromatographic procedure. The modification uses the same reagent formulations as described in the original paper (10), and the protocol is as follows:

1. Pipet 100 µL of serum, control, or standard (50 mg/L) into separate 10 x 75 mm test tubes.

2. Pipet 200 µL of ethyl acetate into each tube and vortex-mix for 15 s.

3. Allow layers to separate and pipet 50 µL of the top layer into each of separate tubes containing 250 µL of acetate buffer (0.3 mol/L, pH 6.0), two drops of 8 mol/L 2,4,6-tri(2-pyridyl)-S-triazine (TPTZ), and one drop of 20 mmol/L FeCl3.

4. Observe the intensity of the blue color after 5 min. Report results according to the following criteria:
   - Color less intense than standard: <50 mg/L
   - Color equal in intensity to standard: 50 mg/L
   - Color of greater intensity than standard: >50 mg/L

We use an aqueous standard rather than a serum-based calibrator because we found that the matrix did not interfere notably. I chose 50 mg/L as the cutoff concentration for this method, to allow classification of the patient into the low-risk or higher-risk categories, as long as the sample is drawn within 10 h of acetyaminophen ingestion. This qualitative procedure is invalid for results <50 mg/L for specimens collected later than this, for which a quantitative procedure is required.

Of 38 commonly used drugs tested, only levodopa, methyldopa, and the phenothiazines interfered. Minor reactions were noted with ascorbic acid, phenylbutazone, and phenylephrine in very high concentrations, which are probably not clinically significant. Salicylate interfered in the qualitative procedure and can be differentiated from acetyaminophen in the quantitative procedure because it forms a rose-colored product (rather than blue). This colored product also forms with salicylate when the 2,4,6-tri(2-pyridyl)-S-triazine reagent is omitted; therefore, this color results from the reaction of ferric chloride with salicylate. Above-normal bilirubin concentrations also reportedly interfere with the quantitative method, but a way to eliminate this interference has been suggested (11).

I find this method to be very reliable. In a retrospective survey of results, I found agreement with chromatographic results in 34 of 36 cases. One of the two exceptions was for a patient whose qualitative result was approximately 50 mg/L and liquid-chromatographic result was 25 mg/L. This patient was psychotic and, although there were no records of it on his chart, may have been taking phenothiazines prescribed by a private physician. In the other case, the respective results were >50 mg/L and 8.4 mg/L, but results by the two methods had agreed for a specimen drawn only 4 h earlier (<50 and 40 mg/L), so laboratory error is a distinct possibility. Unfortunately, this could not be verified because the excess sample was inadvertently discarded.

The physicians at our hospital use this screen primarily to rule out toxic acetyaminophen overdose. Thus, <50 mg/L indicates there is no need for therapy with N-acetylcysteine and the physicians may focus their attention elsewhere. With positive results (>50 mg/L), we suggest that the physician await the quantitative results before initiating therapy. In certain cases, i.e., a very strongly positive reaction with the qualitative test, therapy is begun immediately; however, results are still confirmed as soon as possible by liquid chromatography.

References

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Assessment of Carryover in Discrete Analyzers

To the Editor:

A feature of many discrete analyzers is that the sample is aspirated through a single probe, and the volume taken up varies with the particular analysis to be carried out. Because the sample probe comes into contact successively with different samples, a sample could be contaminated with traces of the one preceding it.

Sample carryover is usually assessed by measuring the effect of an analyte present in plasma in high concentration on results for a succeeding sample of plasma with a low concentration of the analyte. One procedure is to aspirate three successive volumes of a high concentration plasma (H) followed by three identical volumes of low concentration plasma (L1, L2, L3). In our experiments, carryover was calculated as \[ ((L1 - L3) / (H - L3)) \times 100\% \] (modified from ref 1).

In the course of evaluation of a new discrete analyzer (Dacos; Coulter Electronics, Inc.), we found that sample carryover depended critically on whether successive sample volumes were the same or different, so that carryover could be seriously underestimated when assessed conventionally.

During the operation of the Dacos, sample (2-20 µL) is aspirated, then dispensed together with either 120 or
200 μL of diluent. The sample probe is then washed with a further 250 μL of diluent, its outer surface being simultaneously washed with de-ionized water, and finally dried.

To assess carryover, we used three pools of plasma. One was supplemented with creatinine and another with glucose, and a third with urea and creatinine. A fourth specimen was plasma from a patient with above-normal aspartate transaminase (AST) activity. The results are given in Table 1.

Carryover was first measured as described above and found to be negligible. The carryover procedure was then changed so that the volume of the supplemented sample was as large as possible, done by programming the instrument to measure creatinine (sample volume 20 μL) but only in the three supplemented samples. The three unsupplemented samples were assayed as before for the analyte to be assessed. When we did this, CK carryover was increased from 0.004% to 1.4%; AST carryover was increased from 0.03% to about 1%. Smaller analyte molecules did not carry over to this extent; urea carryover remained undetectable and glucose carryover increased from undetectable to about 0.16%.

The efficiency with which the probe is washed between samples depends on several factors: sample volume, the chemical nature of the analyte, the inner surface of the probe, the constituents of the wash solution, and the volume of solution flushing the probe. These factors were not systematically evaluated. We do not judge this degree of carryover to be a drawback except under unusual circumstances, but we think that the modified procedure we have described should be incorporated in the assessment of carryover in discrete analyzers.

When these findings were made known to the manufacturer, the software was modified so that the diluent wash between sample aspirations was increased from 250 to 500 μL. This change decreased CK carryover by approximately a third.

**Reference**


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**Table 1. Effect of Sample Volume on Carryover**

<table>
<thead>
<tr>
<th>Sample vol</th>
<th>Diluent vol</th>
<th>Mean H value</th>
<th>Unsupplemented plasma samples</th>
<th>Carryover, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>L2</td>
<td>L3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>microliter</td>
<td>microliter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST, U/L</td>
<td>6</td>
<td>120</td>
<td>1990</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>14.3</td>
<td>14.1</td>
</tr>
<tr>
<td>CK, U/L</td>
<td>4</td>
<td>120</td>
<td>65000</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>76.4</td>
<td>74.5</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>20</td>
<td>120</td>
<td>1468</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>101.5</td>
<td>102.4</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>4</td>
<td>200</td>
<td>153</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>4.50</td>
<td>4.91</td>
</tr>
<tr>
<td>Urea, mmol/L</td>
<td>2</td>
<td>200</td>
<td>102.3</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>5.38</td>
<td>5.36</td>
</tr>
</tbody>
</table>

(a) Same volume of sampling throughout

(b) High-volume sampling followed by low-volume sampling

All values are the mean of 10 assays. H, high concentration; L, low concentration.

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**Effect of Long-Term Storage on the Assay of Iron and Iron-Binding Capacity in Serum**

To the Editor:

Questions were raised in our laboratory on whether long-term storage affected the ability of transferrin to retain its bound iron and to become fully saturated with additional iron. We report here the results of a longitudinal study in which we evaluated results for lyophilized serum stored at 4 °C and for freshly frozen serum stored at −70 °C, both for 10 to 14 years, with respect to iron content and total iron-binding capacity (TIBC).

Laboratory ware was freed from iron by soaking it overnight in chromic acid cleaning solution, followed by copious rinsing with de-ionized water. All water used in reagent preparation and specimen processing was de-ionized such that its resistance was greater than 7 MΩ (1). Horse serum was used as the source of iron-binding protein. Equine whole blood was collected in iron-free plastic bottles, allowed to clot and centrifuged (1000 × g, 4 °C, 30 min). Serum supernates were pooled, apportioned into 60-mL iron-free plastic bottles, then either lyophilized and stored at 4 °C or promptly frozen and stored at −70 °C.

We measured iron concentration and TIBC in triplicate by the method of the Expert Panel on Iron of the International Committee for Standardization in Hematology (2).

The stability of iron and TIBC in serum stored several months at 4 °C and at household freezer temperature has been described (3). Our study extended this type of investigation to cover a storage period of 10–14 years. Figure 1 summarizes our findings. A comparison of the original values with the results for the stored samples showed a good correlation (r = 0.885; n = 66).

We conclude that if serum is properly preserved, the integrity of transferrin to bind iron remains intact for several years. These findings should be relevant for commercial firms that prepare control sera for clinical and research laboratories. In addition, these data indicate the degree of urgency with which those laboratories involved in analyzing specimens for iron and TIBC need to make such determinations.

We gratefully acknowledge the donation of equine whole blood by the Division of Veterinary Medicine, Walter Reed Army Institute of Research, Washington, DC 20307-5100. The original values for serum iron and total iron binding capacity were kindly provided by Dr. Marcel Conrad, Chief, Division of Hematology, University of Alabama School of Medicine, Birmingham, AL 35292.

**References**

2. International Committee for Standardization in Hematology. Proposed recommen-