In a detailed European collaborative study done some time ago (3) several participating laboratories assayed cortisol in liquid and lyophilized reconstituted human serum by ID-MS. The results (Table 1) show, in accordance with the study carried out by Patterson et al. and others, that lower figures for intralaboratory variance in micromolar concentration can be achieved than previously anticipated and should be quite sufficient for a reference method. Figures for intralaboratory imprecision (CV) as low as 0.14% have been reported for analytes at millimolar concentrations (4). Interlaboratory variance, however, can be quite high, emphasizing the need for strict control of systematic errors and indicating that the quality of reference methods involving ID-MS might be determined by the magnitude of the interlaboratory variance. This probably would also apply to other types of reference methods. One can speculate on the magnitude of allowable interlaboratory variance. There is no simple answer to the question (5). Even interlaboratory CVs of <3% imply that laboratories using a control material assayed by ID-MS can accept any value within an interval of ±6% (95% confidence limit) of the grand mean as being correct.

We would suggest that figures for interlaboratory variance should be provided before a proposed reference method is finally accepted. The newly established International Information System on Reference Technology (Executive Office: IFCC Office for Reference Materials and Methods) should greatly facilitate such a development. The system will help to identify expert laboratories that are willing to participate in transferability studies of ID-MS reference methodology and will provide detailed documentation of analytical procedures, an excellent example of which has been presented by Patterson et al.

Table 1. Assay of Serum Cortisol by ID-MS in a European Collaborative Study

<table>
<thead>
<tr>
<th></th>
<th>A*</th>
<th>B*</th>
<th>C*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interlab. mean value, nmol/L</td>
<td>251</td>
<td>446</td>
<td>812</td>
</tr>
<tr>
<td>Interlab. variance, CV, %</td>
<td>1.3</td>
<td>2.8</td>
<td>5.9</td>
</tr>
<tr>
<td>Interlab. variance, % of mean</td>
<td>0.4-3.0</td>
<td>1.8-2.4</td>
<td>1.3-3.1</td>
</tr>
<tr>
<td>No. labs. participating</td>
<td>4</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>


References


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Enzymic Determination of Cholesterol in Plasma Obtained by Fingerstick

To the Editor:

In recent years, enzymatic methods of cholesterol determination have been developed (1). These easy-to-use methods have provided rapid, reliable cholesterol results on relatively small volumes of venous plasma. It is sometimes desirable, however, to obtain blood by the fingerstick method. Cholesterol determinations on capillary plasma obtained by fingerstick have been validated with gas-liquid chromatography (2). The following study was necessary to determine if capillary plasma can also be analyzed by enzymic methods.

Venous and capillary blood samples were collected from each of 40 fasting adults. Plasma cholesterol was quantified by enzymic methods in a Gilford 3500 analyzer, with reagents supplied by Worthington Diagnostics. Capillary blood was obtained by skin puncture (fingerstick). Each sample was collected into three heparinized microhematocrit tubes. The plasma was transferred to a sample cup and assayed for cholesterol as described above. The means and correlation coefficients were determined for the entire group of 40 subjects and for each quartile.

The correlation between the venipuncture and fingerstick method was .98. The means agreed within 5%. The correlation coefficients for the 1st, 2nd, 3rd, and 4th quartiles were as shown in Table 1.

Although analyses for cholesterol both by gas-liquid chromatographic and enzymic methods have the advantage of requiring a small sample volume, the former is very tedious and analysis time is long. In this study, the high correlation between values for venous and capillary plasma cholesterol indicates that enzymic analysis of capillary plasma is a reliable and rapid alternative to previous methods. This is an advantage when a small sample size is desirable, when the subjects' aversion to venipuncture is great, or when veins may collapse easily or are difficult to find. It has special applicability in large screenings of young children or when repetitive sampling of the same individual is required.

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Mismeasurement of Methemoglobin ("Methemoglobin Revisited")

To the Editor:

A recent review of sulfhemoglobinemia by Park and Nagel (1) referred to a problem that we also have encoun-
tered, namely, mismeasurement of sulfhemoglobin as methemoglobin. A sample of heparinized whole blood from an elderly man was sent to our laboratory from a neighboring hospital. The man was described as appearing blue and was said to have "persistent methemoglobinemia," with concentrations measured by the Co-Oximeter® (Instrumentation Laboratory, Lexington, MA 02173) (2) remaining at 30% during the three days of his hospitalization up to that time. His $pO_2$ ranged from 50 to 60 mmHg. Because he was not in acute respiratory distress, the patient had not been treated with methylene blue.

In our laboratory, spectrophotometric analysis of the blood demonstrated an absorption band at 620 nm, which did not disappear upon addition of dithionite but which did disappear on addition of hydrogen peroxide. This finding is consistent with the presence of sulfhemoglobin (3).

Analysis of the samples for both methemoglobin and sulfhemoglobin by a commonly used modification of the Evelyn–Malloy (4) method, with use of a scanning visible-range spectrophotometer, yielded values of 3% and 14% of total hemoglobin, respectively. In this case the Co-Oximeter mismeasured sulfhemoglobin (which persists for the lifetime of the erythrocyte) as methemoglobin, resulting in the diagnosis of "persistent methemoglobinemia." The Evelyn–Malloy procedure involves absorbance measurement at 630 nm before and after adding neutralized cyanide, but in the Co-Oximeter procedure a single measurement is used and the procedure will be perturbed by nonmethemoglobin compounds having absorbing at or near 630 nm (e.g., sulfhemoglobin at 620 nm). The manufacturer acknowledges this problem (2).

We have also encountered further difficulties with the analysis for methemoglobin. In our institution another patient with methemoglobinemia received 1 mg of methylene blue per kilogram body weight in the emergency room. Twelve hours later the methemoglobin concentration as measured in the Co-Oximeter was still high (30%), while analysis by the method of Evelyn–Malloy showed no measurable amount of methemoglobin. Studies in our laboratory revealed that, for the same reason as described here, methylene blue will also be mismeasured by the Co-Oximeter as methemoglobin. This too has been reported by the manufacturer (2).

Although many laboratories recommend freezing blood samples if a delay in methemoglobin analysis is anticipated, our experience indicates that such action, in fact, produces methemoglobinemia. Blood samples with no detectable amount of methemoglobin were stored frozen (−20 °C) for 24 h. After thawing, the samples on re-analysis were found to contain up to 14% methemoglobin. For samples containing methemoglobin to start with, the effect of freezing was even more pronounced. Three blood samples containing methemoglobin concentrations of 21% to 25% were stored frozen for 24 h, thawed, and reanalyzed. Methemoglobin concentrations now ranged from 53% to 58% of total hemoglobin. In contrast, refrigeration at 4 °C had little or no effect on methemoglobin concentration. Although this observation was reported in 1966 (5), it apparently has not received widespread attention.

Finally, we have noted that sample collection per se can interfere with methemoglobin analysis. Blood from normal volunteers drawn into EDTA-containing or heparinized glass blood-collection tubes had no measurable amounts of methemoglobin; a specimen of the same blood, in sodium fluoride/potassium oxalate blood-collection tubes for 1 h, yielded methemoglobin concentrations of 3%. Samples already containing methemoglobin also demonstrated concentration increases, being 30% in EDTA-treated and heparinized samples but 37% in fluoride/oxalate-anticoagulated specimens. To our knowledge, this observation has not previously been reported.

Laboratory personnel and physicians alike should be aware that methemoglobin may be mismeasured by the Co-Oximeter whenever sulfhemoglobin is present or whenever methylene blue (the treatment for methemoglobinemia) has been administered. We recommend that in these situations the spectrophotometric Evelyn–Malloy procedure (4), or a modification of it, be used. We also caution against analysis for methemoglobin in blood samples that have been frozen or that have been collected into fluoride/oxalate anticoagulant. Mismeasurement of methemoglobin may have profound clinical consequences.

References

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Effect of Silicone-Separator Tubes and Storage Time on Ionized Calcium in Serum

To the Editor:

The need for a rapid, complete separation of serum from the clot has led to the introduction of "silicone-separator gel" (SST) tubes (1, 2). These tubes not only contain a silicone gel for the separation, but also silica particles to facilitate clotting (3). It is essential that such tubes not add or adsorb components that affect results for ionized calcium (Ca²⁺) determinations, such as calcium or hydrogen ions or calcium-complexing agents such as lactate. Recently, Toffaletti et al. (4) reported consistently higher (by 0.02 mmol/L) Ca²⁺ concentrations in samples from SST tubes than in those from plain Vacutainer Tubes (both from Becton Dickinson Inc., Rutherford, NJ 07070), a difference explained by a lower initial pH in the SST tubes. Because the observed difference was not fully clarified, we wanted to investigate this difference further and also to obtain more information on the effect of storing samples at 4 °C for longer periods.

Blood was sampled from 34 healthy volunteers (ages 18–45 years) into one 10-mL plain Vacutainer Tube and one 10-mL SST tube for each person. After clotting, the samples were stored at 4 °C and analyzed within 3 h with a Radiometer ICA 1 (Radiometer A/S, DK-2400 Copenhagen, Denmark). The results are shown in Table 1.

As can be seen there was a small, statistically significant, but not clinically significant, difference for Ca²⁺, pH, and ionized calcium corrected to pH 7.40 (Ca²⁺ 7.40) in the paired samples. This observation may be explained in several ways: (a) Some CO₂ is always lost into the gaseous phase of the tube, in an amount that may differ between the two kinds of tubes. (b) A bias related to sampling all of the plain tubes before any of the SST tubes may occur as an effect of stasis (5). However, by these explanations there should not