Plastic versus Glass Tubes: Effects on Analytical Performance of Selected Serum and Plasma Hormone Assays, Carol M. Preisnser, William M. Reilly, Richard C. Cyr, Dennis J. O’Kane, Ravinder J. Singh, and Stefan K.G. Grebe (Endocrine Laboratory, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN; * address correspondence to this author at: Endocrine Laboratory, Hilton 730C, Mayo Clinic, 200 1st St, SW, Rochester, MN 55905; fax 507-284-9758, e-mail grebs@mayo.edu)

During the last decade, plastic blood collection tubes have been progressively replacing glass tubes. Plastic tubes are not only less expensive but also safer than glass tubes, because they are less likely to break. Unfortunately, it is frequently difficult or impossible for individual laboratories to obtain comprehensive data on the equivalence of replacement plastic tubes vs their original glass counterparts. This is a particularly important issue for many endocrine assays, especially peptide hormones. These often degrade rapidly and can adsorb to a variety of surfaces (1–7). Changing from glass to plastic tubes can also be problematic for analytes that are regarded as stable. An example of the latter can be seen in therapeutic drug monitoring, where plastic tubes have been shown to influence the measured concentrations or stabilities of several drugs (8–10). Similar concerns may apply to low-molecular-weight hormones, such as steroid hormones and biogenic amines. These are increasingly assayed by HPLC, gas chromatography–mass spectrometry, or liquid chromatography–tandem mass spectrometry. It is conceivable that low-molecular-weight organic substances released by plastic tubes could interfere in some of these assays (11) and that small changes that are not detected by immunoassays would change the results of more specific methods. Finally, in certain situations, such as serial monitoring of tumor markers, even minor discrepancies between glass and plastic tubes may gain significance during changeover from one type of collection to the other.

We designed the present study to give a reasonable representation of the range of analytes and analytical methodologies used in our laboratory, with particular emphasis on peptide hormones because of their known instability. We selected the following analytes:

(a) Five peptide hormones—adrenocorticotropic (ACTH), insulin, insulin-like growth factor I (IGF-I), parathyroid hormone (PTH), and human growth hormone (hGH)—covering a range of known stabilities, physicochemical properties, and molecular weights;

(b) Two glycoproteins, human β-chorionic gonadotropin (β-hCG) and cancer antigen-125 (CA-125), an ovarian cancer tumor marker; and

(c) Two steroid hormones, cortisol and 17-hydroxyprogesterone (OHPG).

The OHPG assay is an in-house, extracted liquid chromatography–tandem mass spectrometry method with an analytical range of 0.33–330 nmol/L, interassay CV of 2.6–10%, and recoveries of added analyte and dilution linearity of 96–118%. The PTH assay is an in-house intact molecule manual direct chemiluminometric assay that uses a pair of in-house-produced polyclonal antibodies with <30% cross-reactivity with PTH(1–34) and no cross-reactivity with more C-terminal fragments. Assay CVs across the analytical range were 5–18%. The other assays are automated direct immunoassays performed on the Beckman-Coulter Access platform (insulin, β-hCG, cortisol, and hGH), the Nichols Advantage system (ACTH and IGF-I), and the Ortho-Diagnostic Vitros ECI-analyzer (CA-125). All automated assays conformed to the respective manufacturers’ performance specifications, which we verified in house. The β-hCG assay uses a combination of a mouse monoclonal antibody to the β-subunit and a polyclonal alkaline phosphatase-linked rabbit anti-total hCG antibody for analyte detection, and goat anti-mouse-antibody-coated paramagnetic beads for separation. The limit of quantification is 0.5 IU/L.

We used a randomized schedule of tube order, generated by our statistics department, for all blood draws to control for any draw-order bias. For all analytes except ACTH, we drew blood from 25 volunteers (age range, 24–57 years; 10 males and 15 females) into serum separator (SS) glass tubes (Becton Dickinson) and into SS plastic tubes manufactured by Becton Dickinson (Vacutainer Tube; designated as type A), Monoject (Sherwood Corvac tube; designated as type B), and Greiner (Greiner Vacuette tube; designated as type C). Except for the insulin hemolysis and ACTH portions of the study (see below), all tubes had the same lot numbers. We froze an aliquot from each tube at −20 °C and kept the remainder at 4 °C for 1 week before also freezing it. All aliquots were thawed and...
analyzed simultaneously. No particular attention was paid to the order of analysis. In addition, we performed experiments with serum pools of β-hCG and CA-125, because the majority of the volunteers’ samples contained low analyte concentrations. We prepared 10 pools (>4 mL), each with various concentrations of β-hCG and CA-125, respectively, that covered the reference interval and included increased concentrations. We distributed each pool equally into four tubes: one glass and three plastic. The tubes were rotated for 30 min at room temperature and then, without any further manipulations, aliquoted and stored as described above. For insulin measurements, we collected samples from an additional 33 donors on a separate occasion into glass and type A plastic tubes. These samples, plus another 46 glass and plastic tube samples (of types A, B, and C) from two different laboratories, were tested for hemolysis of the separated serum by automated hemoglobin assay (Hitachi 747 chemical analyzer hemolysis index function). Significant hemolysis was defined as a serum hemoglobin >100 mg/L because this appears to be the lowest extent of hemolysis that has been shown to lead to falsely low serum insulin determinations (12).

For ACTH, we collected samples from 31 volunteers into siliconized EDTA glass tubes, ordinary EDTA glass tubes, and type A EDTA plastic tubes. The other plastic tube brands were omitted because they had not shown any substantial differences from type A plastic tubes in the other assays (see below). We froze the aliquots immediately or after 4 or 24 h at 4 °C. Again we analyzed all aliquots at the same time.

Continuous data were compared by nonparametric statistics (Fisher paired-sign test) or by ANOVA for repeated measures (ACTH only). Categorical data (significant hemolysis) were compared by χ² analysis. All P values were adjusted for multiple comparisons.

No differences were seen between glass and plastic SS tubes for cortisol, OHPG, hGH, and β-hCG (see Table 1 in the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol50/issue7/). There were isolated statistically significant differences between glass and plastic SS tubes after 7 days of storage for insulin, IGF-I, and PTH (Table 1). These differences were small and clinically insignificant. CA-125 concentrations decreased over time in all sample tubes, indicating a degree of general storage instability. In addition, the CA-125 results differed increasingly over time for all brands of plastic tubes compared with glass tubes. However, these differences were, for the most part, not considered clinically significant. Results obtained with plastic tubes B and C displayed a tendency toward increased variability compared with glass tubes and type A plastic tubes.

Visible hemolysis was observed in several insulin samples drawn into glass tubes, but none was observed in the plastic tubes. Because hemolysis can lead to falsely low insulin measurements (1, 4, 12), this phenomenon was investigated further in a larger sample series. The mean hemoglobin concentrations (67 vs 44 mg/L; median, 50 vs 30 mg/L; P <0.02) and the proportions of samples with significant hemolysis (18% vs 5.3%; P <0.013) were higher in glass tubes than in type A plastic tubes. Type B and C plastic tubes, which had been used in addition to type A tubes in the samples drawn in the two other laboratories, did not differ from type A tubes and also displayed less extensive hemolysis than glass tubes.

ACTH was unstable at 4 °C in all three EDTA sample tubes, but there was no difference between siliconized glass tubes, ordinary glass tubes, and type A plastic tubes (Fig. 1).

We conclude that plastic gel barrier tubes manufactured as Becton Dickinson Vacutainer, Sherwood Corvac

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<th>Table 1. Comparison of glass and plastic tubes for analytes that displayed at least one significant difference. a</th>
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<td><strong>Median (minimum, maximum) values for analytes</strong></td>
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a The full result set, including the results for analytes without any differences between glass and plastic tubes (hGH, β-hCG, cortisol, and OHPG), can be viewed in Table 1 in the online Data Supplement.

b Combined data from 25 volunteers and 10 serum pool experiments.

c P <0.002.
d P <0.0003.
Monoject, and Greiner Vacutette are comparable to Becton Dickinson glass SS tubes when used to collect specimens for endocrine testing. There may be minor differences among the three brands, but they are unlikely to be clinically significant. Some analytes, such as ACTH, are inherently unstable, but this is not accentuated by plastic tubes. Conversely, there is also no evidence for the widely held belief (quoted in the specimen requirement instructions of many laboratories) that significant amounts of analytes that could be affected similarly or worse, and may behave differently at higher analyte concentrations.

Finally, plastic is the better specimen container to reduce interference from hemolysis in clinical testing, which is particularly relevant to insulin measurements but may affect other tests.

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References

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Assessment of Parathyroid Function in Clinical Practice: Which Parathyroid Hormone Assay Is Better? Stefano A. Santini,1 Cinzia Carrozza,1 Carlo Vulpino,2 Ettore Capoluongo,1 Giovanna Luciani,2 Paola Lulli,1 Bruno Giardina,1 and Cecilia Zuppi1* (1 Institute of Biochemistry and Clinical Biochemistry, Hormone Research Unit, and 2 Institute of Surgical Clinic, Hemodialysis Unit, Catholic University School of Medicine, Rome, Italy; * address correspondence to this author at: Institute of Biochemistry and Clinical Biochemistry, Catholic University School of Medicine, Largo F. Vito 1, 00168 Rome, Italy; fax 039-6-30151918, e-mail czuppi@rm.unicatt.it)

Parathyroid hormone (PTH) is a single-chain 84-amino acid polypeptide synthesized by the parathyroid glands. In the blood it is thought to circulate as a mixture of whole molecule [PTH (1–84)] and N- and C-terminal (C-PTH) fragments produced in the parathyroid glands and liver (1, 2). In patients with intact renal function, the non-(1–84) PTH, identified by HPLC, reportedly accounts for ~21% of PTH(1–84) in hypercalcemia and ~10% in hypocalcemia (3). C-PTH fragments accumulate in renal failure up to 40–50% of total PTH (4) and may be implicated in the PTH resistance observed in these patients. It is not known whether these fragments can mimic the biological effects of PTH(1–84) or, in contrast, react with distinct receptors (5–8).

The major large C-PTH fragment with partially preserved N-terminal structure is PTH(7–84), often considered to be the likely cross-reacting peptide in “intact PTH” (I-PTH) assays (6–9). The biological activity of this...