than polyclonal antibodies were involved and that its continuing manifestation over the time period is dependent on a supply of antisera with constant behavior toward ceruloplasmin. Nevertheless, we find no better explanation. The phenomenon cannot be ascribed to changes in calibrators or the primary standard because we describe a temporal effect that occurs subsequent to the preparation and distribution of each new serum pool. As 17 new pools were prepared and circulated during the period under study, approximately one each month with new and aged pools constantly overlapping, such an alternative explanation is not tenable.

This phenomenon is new, having been described more than 20 years ago and ascribed to the process described above (3). The exact mechanism remains to be elucidated, but our preliminary experiments (not shown here) using isoelectric focusing suggest that it is not attributable to alterations in glycosylation with age; we are investigating further the lability of ceruloplasmin with respect to copper binding and proteolytic degradation (4).

Overall, the importance of our findings is that a universal reference interval based on a calibrant traceable to CRM 470 clearly cannot be used for ceruloplasmin in fresh specimens from patients.

References

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Comparison of Serum and Heparin-Plasma Samples in Different Generations of Dimension Troponin I Assay

To the Editor:

An editorial in the June issue of the Journal pointed out the usefulness of plasma instead of serum for cardiac troponin I (cTnI) determinations. However, because some analytical systems yield significant differences between serum and plasma troponin concentrations, the author of the editorial advocates more peer-reviewed studies before new assays are implemented in hospital-based laboratories (1). In September 2001 and March 2002, we carried out two comparison studies of cTnI measurements in plasma and serum samples, using different generations of the same assay (Cardiac Troponin-I Flex; Dade Behring). Both serum and plasma samples have been reported as suitable for cTnI determinations in the first (2) and second (3) generations of the assay. We collected two different series of 39 blood samples from patients admitted to the intensive care unit of our hospital during each month. For each patient, a serum (Venoject II, Autosep Gel + Act Z; Terumo Europe) and a plasma specimen (Venoject II, Autosep Gel + lithium heparin, 75 IU/tube) were obtained. cTnI was measured in duplicate in both series with reagents that came with the Cardiac Troponin-I Flex reagent (batch BD1349 in September 2001 and batch CK3031 in March 2002) and cTnI calibrators (batches OMD095 and IHD045, respectively) on the Dimension RxL Analyzer (Dade Behring). Linear regression analysis of the September 2001 results produced a y-intercept of 0.389 µg/L (SE 0.032 µg/L) and a slope of 0.978 (0.014). The correlation was good (r = 0.9977; 95% confidence interval (CI), 0.9956–0.9988; P < 0.0001). Results for serum [median cTnI value (range), 4.08 (0.30–619.99) µg/L] and plasma [5.50 (0.30–57.58) µg/L] did not show a significant difference (Wilcoxon rank-sum test for paired data).

Linear regression analysis of the March 2002 results yielded a y-intercept of −0.125 µg/L (SE, 0.0216) and a slope of 0.941 (0.010). The correlation was good (r = 0.9977; 95% CI, 0.9956–0.9988; P < 0.0001). However, results for serum [median cTnI value (range), 5.35 (0.015–77.95) µg/L] and plasma [5.00 (0.01–74.71) µg/L] showed a significant difference (Wilcoxon rank-sum test for paired data, P < 0.001). The assay used in September had a mean bias for plasma samples of 1% (95% CI, −2.4% to 4.45%) compared with paired serum samples, whereas the second-generation assay had a mean bias for plasma samples of −11.4% (95% CI, −17.53% to −5.26%). It appears noteworthy that only three pairs of samples had differences >20% (29.5%, 28.3%, and 24.1% at cTnI concentrations of 1.77–4.08 µg/L) in the first-generation assays, whereas seven pairs of samples had differences >20% with the second-generation Dimension assay (68%, 62.5%, 50%, 43.5%, 33.3%, 30.2%, and 26.4%); five at concentrations <0.2 µg/L, one at 0.7 µg/L, and 1 at ∼10 µg/L.
Fig. 1 confirms the point made by Dewitte et al. (4) that the uncritical adoption of Bland–Altman plots, far from clarifying, blurs the obtained results. As seen in panels A and B of Fig. 1, the first-generation, not the revised, Dimension cTnI assay yielded consistent results for serum and plasma. After logarithmic transformation of the $x$ data and construction of a $y$ axis using percentage values (Fig. 1, C and D), the results obtained for plasma and serum become more consistent. The evaluation steps suggested by international scientific bodies, e.g., IFCC, including anticoagulant validation, should be carried out even when an assay is “simply” reformulated by the manufacturer, and results should be carefully assessed. We agree that caution is required when cTnI results are obtained in different matrices (1, 5); however, heparinized plasma and serum samples appear interchangeable in cTnI measurement with the second-generation Dimension RxL, as can be demonstrated when properly investigated as suggested by Dewitte et al. (4). Finally, even a much smaller group of samples, some of which contained very low concentrations, yielded results similar to those obtained for a much larger group of samples (3, 5).

References

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Falsely Increased Free Triiodothyronine in Sera Stored in Serum Separator Tubes

To the Editor:

We recently encountered inappropriately increased free triiodothyronine (FT₃) concentrations when blood specimens were collected in glass separator tubes (SST™, Vacuette®). Although Banfi and Pontillo (1) reported no differences between plain and gel-containing tubes until 72 h after blood collection for FT₃, we investigated the effects of storing specimens in gel-containing tubes on these analytes.

We collected blood specimens from 11 healthy volunteers in both SST (16 × 100 mm) and plain glass (13 × 100 mm) Vacutainer Tubes. All specimens were allowed to clot for 30 min at room temperature before centrifugation at 1300 g for 10 min. Sera separated in SST tubes remained on the separator gel, whereas sera separated in plain tubes were transferred into 13 × 100 mm plain tubes (Vacutainer) for storage. All SST specimens and transferred control specimens were kept capped and stored in the same rack in the refrigerator. We measured TSH, FT₄, and FT₃ in duplicate on an Immulite 2000 analyzer with reagents from the manufacturer (Diagnostic Products Corporation) at 40 min (0 h), 24 h, 48 h, and 72 h after drawing. We used arithmetic means of the duplicates for statistical analysis. The significance of differences between baseline analyte means of the groups was assessed by the Student paired t-test.

Among the three analytes studied, only FT₃ showed significant differences between the SST and plain tubes (Table 1). Repeated-measures ANOVA for FT₃ showed a significant group effect (F = 98.2; P < 0.001), time effect (F = 9.449; P < 0.001), and group–time interaction (F = 12.7; P < 0.001). Initial FT₃ values of the sera in SST tubes were significantly higher than those in plain tubes (t = 3.8; P = 0.005). The within- and between-run CVs for FT₃ were 5.4% and 6.0%, respectively (n = 21 for both), at a concentration of 6.1 pmol/L.

To investigate the mechanism of the FT₃ increase, we pipetted 3.0 mL of saline into SST and plain tubes (n = 2) and measured FT₃ in duplicate in these tubes at the same time points. All of the results were <1 pmol/L, but cps values for FT₃ in SST tubes decreased gradually with time (data not shown). To confirm this finding, we added the “high adjustor” of the Diagnostic Products Corporation FT₃ reagent set to saline to give a physiologic concentration of T₃ in SST and plain tubes (n = 2) and measured FT₃ during storage at 4 °C (Table 1).

We carried out an additional experiment with newly drawn specimens from 11 healthy individuals. After the sample collection and pro-

Table 1. Summary of data obtained in experiments.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Tubes</th>
<th>Means (SDs) at time points</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
<td>2 h</td>
</tr>
<tr>
<td>Samples at 4 °C (n = 11): Immulite 2000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FT₃, pmol/L</td>
<td>SST 4.99 (0.78)</td>
<td>5.67 (0.84)</td>
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<tr>
<td></td>
<td>Plain 4.15 (0.92)</td>
<td>4.31 (0.82)</td>
</tr>
<tr>
<td>FT₄, pmol/L</td>
<td>SST 17.0 (1.72)</td>
<td>16.87 (1.32)</td>
</tr>
<tr>
<td></td>
<td>Plain 16.72 (1.02)</td>
<td>16.87 (0.90)</td>
</tr>
<tr>
<td>TSH, mIU/L</td>
<td>SST 1.030 (0.764)</td>
<td>1.055 (0.801)</td>
</tr>
<tr>
<td></td>
<td>Plain 1.023 (0.750)</td>
<td>1.023 (0.731)</td>
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<td>Saline + calibrator (n = 2): Immulite 2000</td>
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<tr>
<td>FT₃, pmol/L</td>
<td>SST 6.52</td>
<td>8.90</td>
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<tr>
<td></td>
<td>Plain 6.18</td>
<td>5.61</td>
</tr>
<tr>
<td>Instrument comparison (n = 11): samples at 4 °C</td>
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<td></td>
</tr>
<tr>
<td>FT₃, pmol/L; Immulite 2000</td>
<td>SST 5.31 (0.85)</td>
<td>5.94 (0.94)</td>
</tr>
<tr>
<td></td>
<td>Plain 4.62 (1.04)</td>
<td>4.74 (0.88)</td>
</tr>
<tr>
<td>FT₃, pmol/L; ACS 180 Plus</td>
<td>SST 4.82 (0.56)</td>
<td>4.43 (0.45)</td>
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<td></td>
<td>Plain 4.86 (0.58)</td>
<td>4.49 (0.47)</td>
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<tr>
<td>T₃ in Immulite 2000, nmol/L (n = 11)</td>
<td>SST 1.57 (0.24)</td>
<td>1.70 (0.23)</td>
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<tr>
<td></td>
<td>Plain 1.54 (0.16)</td>
<td>1.66 (0.23)</td>
</tr>
<tr>
<td>Tube comparison (n = 6): FT₃, pmol/L</td>
<td>SST 5.31 (0.73)</td>
<td>6.35 (1.06)</td>
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<tr>
<td></td>
<td>Vacutte 5.02 (0.71)</td>
<td>5.10 (0.42)</td>
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