ter about the regression line (5, 7, 8). Regression formulas and correlation coefficients ($r^2$) appear to be the sole means by which data were interpreted with little attention to scatter. An incomplete picture of the comparative data can result from these omissions. Moreover, Turpeinen et al. (5) report that “several samples, however, displayed very large differences” between Diasorin RIA and their HPLC method. Mausell et al. (6) report significant scatter in the Bland–Altman plot encompassing a range of +25 to −40 nmol/L (+10 to −16 μg/L) for Diasorin RIA vs LC-MS/MS. These results are much in line with our report of an $\text{Sy/x} \sim 18$ nmol/L (7.3 μg/L) for HPLC vs Diasorin RIA. Furthermore, the Binkley study (4) that Dr. Schmidt refers to describes the discordant results among laboratories and methods for the determination of 25(OH)D. Their data do not allow us to draw any conclusions about the accuracy of the Diasorin method. Overall, our results are not significantly different from the data reported by other investigators.

The International Vitamin D External Quality Assessment Scheme (DEQAS) proficiency survey (9) is a valuable resource for assessing peer performance in measuring 25(OH)D and is based on how close a laboratory’s test result is to the all-laboratory trimmed mean. Unfortunately, the receipt of a passing grade does not scientifically support a method as being accurate and could give a false sense of security. The DEQAS program describes the relative performance among participants and, unless specifically controlled with traceable accurate reference standard materials for 25(OH)D$_3$ and 25(OH)D$_2$, such assessments present only a relative indication of performance. Accordingly, Dr. Schmidt’s judgment that the Diasorin RIA is accurate based on favorable DEQAS results is unwarranted and an overinterpretation of the proficiency testing data. We, too, have received passing grades. Acceptable performance in proficiency testing programs is not necessarily a measure of accuracy. A global effort to produce and use serum-based standard materials for 25(OH)D$_3$ and 25(OH)D$_2$ is essential to properly challenge all methods for accuracy. Currently, NIST is in the process of producing these standard materials.

Finally, we applaud Diasorin for taking steps to produce controls that should better describe the comparative performance of their 25(OH)D RIA and LIAISON$^\text{TM}$ immunoassays, and we look forward to the published results.

References

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Size-Exclusion Chromatography of Circulating Cardiac Troponin T

To the Editor:
In a recent issue of Clinical Chemistry, Fahie-Wilson et al. (1) reported their use of gel-filtration chromatography and size-exclusion chromatography (SEC), combined with off-line measurement, to investigate circulating forms of cardiac troponin T (cTnT). We believe that the presented data did not justify the main conclusion of the paper, that cTnT circulates predominantly in the free, intact form in serum of patients with kidney failure and patients with acute coronary syndrome.

Behavior of proteins in SEC is determined mainly by their hydrodynamic volume (expressed as Stokes radius, R$_S$). Nonglobular proteins are known to have a larger R$_S$ than globular proteins with a comparable relative molecular mass (M$_r$), and cTnT is far from globular (2). Therefore, as is generally agreed, the separation of the nonglobal cTnT protein in an SEC column is better described with the R$_S$ than the M$_r$ (3). Hence, we used R$_S$ to analyze the data from Fahie-Wilson et al. (1).

To calculate the R$_S$ of the immuno-reactive peaks, we deduced the K$_w$ of the calibrator proteins from Fig. 1 of the Fahie-Wilson article (ribonuclease A, 0.393; chymotrypsinogen A,
The void volume ($V_0$) of the column was estimated from the reported column volume ($V_t$) of 75 mL and from the elution volume ($V_e$) and $K_{av}$ of the globular proteins albumin ($V_e = 31.4$ mL; $K_{av} = 0.118$) and prolactin ($V_e = 40.2$ mL; $K_{av} = 0.280$). Entering these numbers into the equation for $K_{av}$:

$$K_{av} = \frac{V_e - V_0}{V_1 - V_0},$$

yielded a $V_0$ of 25.1 mL (albumin) and 26.4 mL (prolactin). The mean of 25.8 mL was used as an estimate of the true $V_0$.

We then calculated the $K_{av}$ for the 2 cTnT reactive peaks from Fig. 2D of the Fahie-Wilson article (peak 1, $V_e = 30.5$ so $K_{av} = 0.09$; and for peak 2, $V_e = 37.0$ so $K_{av} = 0.22$). When these $K_{av}$ were entered into equation 1, Stokes radii of 37 Å and 26 Å were obtained. In addition, we used equation 1 to calculate the highest $R_s$ ($K_{av} = 0$) that could be separated with the column ($R_{s,\ max} = 48$Å). We believe these $R_s$ do not correspond to the troponin complex and free cTnT. The $R_s$ of the troponin complex has been determined to be 57Å (5). Hence, we may conclude that the first peak cannot correspond to the troponin complex, because this would have eluted at $V_0$. Unfortunately, the $R_s$ of cTnT has never been determined.

It is plausible, however, that the first peak corresponds to intact free cTnT. Based on the $R_s$ of bovine TnC ($M_r \approx 18 400$) and cTnT ($M_r \approx 23 900$) (24Å and 30Å, respectively (6), it is likely that the larger cTnT ($M_r \approx 34 500$) has an $R_s$ of 37Å. The online SWISS-PROT database indicated 85%, 89%, and 99% homology between bovine and human cardiac troponin T, I, and C, respectively. The second peak would then correspond to an immunoreactive fragment. Interestingly, a small immunoreactive fraction just before 30 mL is visible in Fig. 2C and corresponds to the first peak from Fig. 2D. This peak could indicate a small amount of the intact protein present in acute coronary syndrome patients. Importantly, because no secondary confirmation was provided (e.g., sodium dodecyl sulfate-polyacrylamide gel electrophoresis or amino acid sequencing), the exact identity of the peaks remains unclear.

In studies such as this, it is essential to use a fully characterized troponin TIC complex standard. Usually, it can be expected that the claims from suppliers are correct and thoroughly checked. However, when we contacted the supplier (SCIPAC), they were not able to provide data showing both intact free cTnT and TIC complex in their standard.

In summary, the conclusions drawn from fundamentally incorrect interpretations of SEC data, without any secondary confirmation, and the use of a troponin complex standard that is not properly characterized may have been misleading to readers. It is more plausible that the correct conclusion from the study by Fahie-Wilson et al. (1) is that no intact free cTnT is present in patients with kidney failure, but that a smaller immunoreactive cTnT fragment is present.

References


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To the Editor:

In contrast to Van Dieijen-Visser’s group, who concluded that in patients with kidney failure the presence of fragments of circulating cardiac troponin T (cTnT) led to increased cTnT concentrations (1, 2), our studies led us to conclude that the increased concentrations were attributable to free, intact cTnT (3). Michielsen et al. suggest that our conclusion is erroneous because we used a partially purified cardiac troponin (cTn) standard preparation that was not properly characterized and because the analysis of our gel filtration chromatography (GFC) results was incorrect.

With respect to the first point of Michielsen et al., we used a commercially available extract of human heart tissue containing cTn at high concentration (SCIPAC Ltd.; product code 184–4). The suppliers had made no claims regarding the relative abundance of troponin forms within this material. However, native–polyacrylamide gel electrophoresis and Western blot analysis of the batch of material we used indicated that the majority of the cTn present was of a molecular mass consistent with the ternary cardiac troponin T-I-C (cTnT-I-C) complex (personal communication, Simon Packer, July 10, 2006).

0.310; ovalbumin, 0.165) and constructed a new calibration curve ($K_{av}$ vs ln $R_s$). To construct this curve we used the Stokes radii of these proteins: 16.4 Å, 20.9 Å, and 30.5 Å, respectively (4). This calibration curve is represented by equation 1:

$$K_{av} = -0.369 \ln(R_s) + 1.427;$$

$$R^2 = 0.999.$$