

Clinical Chemistry
and Hematology Laboratory
Hospital of Verona
Piazzale A. Stefani 1
37126 Verona, Italy

*Author for correspondence. Fax 39-045-8072156; e-mail romolo.dorizzi@mail.azosp.vr.it.

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 serum separator tubes (SSTTM; Vacutainer[®]). Although Banfi and Pontillo (1) reported no differences between plain and gel-containing tubes until 72 h after blood collection for FT₃ [and thyrotropin (TSH) and free thyroxine (FT₄)], 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 1300g 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 and within groups was analyzed by repeated-measures ANOVA. 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.

Experiment	Tubes	Means (SDs) at time points			
		0 h	2 h	4 h	72 h
Samples at 4 °C ($n = 11$); Immulite 2000	FT ₃ , pmol/L	SST 4.99 (0.78)	5.67 (0.84)	6.41 (1.11)	6.13 (0.77)
	Plain	4.15 (0.92)	4.31 (0.82)	4.30 (0.88)	3.91 (0.73)
FT ₄ , pmol/L	SST	17.0 (1.72)	16.87 (1.32)	17.57 (1.68)	16.36 (1.81)
	Plain	16.72 (1.02)	16.87 (0.90)	17.49 (1.30)	16.20 (1.37)
TSH, mIU/L	SST	1.030 (0.764)	1.055 (0.801)	0.982 (0.750)	1.064 (0.817)
	Plain	1.023 (0.750)	1.023 (0.731)	1.002 (0.734)	1.036 (0.755)
Saline + calibrator ($n = 2$); Immulite 2000	FT ₃ , pmol/L	SST 6.52	8.90	10.04	10.86
	Plain	6.18	5.61	5.44	5.44
Instrument comparison ($n = 11$); samples at 4 °C	FT ₃ , pmol/L; Immulite 2000	SST 5.31 (0.85)	5.94 (0.94)	6.63 (1.24)	6.45 (0.95)
	Plain	4.62 (1.04)	4.74 (0.88)	4.84 (0.94)	4.43 (0.96)
FT ₃ , pmol/L; ACS 180 Plus	SST	4.82 (0.56)	4.43 (0.45)	3.86 (0.43)	3.80 (0.44)
	Plain	4.88 (0.58)	4.49 (0.47)	3.98 (0.47)	3.83 (0.44)
T ₃ in Immulite 2000, nmol/L ($n = 11$)	SST	1.57 (0.24)	1.70 (0.23)	1.81 (0.31)	1.93 (0.26)
	Plain	1.54 (0.16)	1.66 (0.23)	1.69 (0.26)	1.91 (0.25)
Tube comparison ($n = 6$); FT ₃ , pmol/L	SST	5.31 (0.73)	6.35 (1.06)	6.62 (1.13)	6.83 (0.90)
	Vacurette	5.02 (0.71)	5.10 (0.42)	5.21 (0.56)	5.26 (0.90)

cessing as mentioned above, we measured FT₃ by Immulite 2000 and ACS 180 plus (Bayer Corporation), as an alternative instrument, and triiodothyronine (T₃) only by Immulite 2000 (Table 1). A significant group effect was shown by repeated-measures ANOVA in SST results obtained by Immulite 2000 and ACS 180 plus ($F = 107.25$; $P < 0.001$). T₃ values gradually increased in both types of tubes with time, and repeated-measures ANOVA showed a significant time effect ($F = 8.450$; $P < 0.001$).

Additionally, we compared two types of evacuated gel-containing tubes, Vacutainer (SST) and Vacuette (16 × 100 mm; Greiner) for FT₃ on the Immulite 2000 ($n = 6$). FT₃ was higher in the SSTs than in the Vacuettes (Table 1). A significant group effect was shown by repeated-measures ANOVA ($F = 4.320$; $P = 0.044$).

Finally, to determine the magnitude of the effect of relatively short-term storage at room temperature on FT₃, we measured FT₃ by Immulite 2000 at 4-h intervals up to 12 h. Repeated-measures ANOVA showed a significant group effect ($F = 34.809$; $P < 0.001$) but not a significant time effect or group-time interaction ($P > 0.05$).

Although the mechanism of the FT₃ increase remains unresolved, the following mechanisms could be envisioned: (a) in gel-containing tubes, the barrier gel could displace FT₃ from binding proteins such as thyroxine-binding globulin, albumin, and transthyretin; (b) the barrier gel could enhance binding of labeled analog to binding proteins; and (c) the barrier gel could show FT₃-like activity or interfere with the indicator chemiluminescent reaction under assay conditions.

This work was supported in part by BIODPC, the distributor of Diagnostic Products Corporation products in Turkey.

Reference

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Aytül Şadan Kılınc
Ahmet Düzoylum
Cenk Fırat Uncugil
Doğan Yücel*

Biochemistry Laboratory
SB Ankara Education
and Research Hospital
Ankara 06340, Turkey

*Address correspondence to this author at: Biochemistry Laboratory, SB Ankara Eğitim ve Araştırma Hastanesi, Ankara 06340, Turkey. Fax 90-312-3621857; e-mail doyucele@yahoo.com.

More Accurate Alternatives to Serum Creatinine for Evaluating Glomerular Filtration Rate

To the Editor:

The timely reports by Laterza et al. (1) and Filler et al. (2) in the same issue of the Journal discuss the use of serum analytes other than creatinine as surrogates for glomerular filtration rate (GFR). They describe the advantages as well as the limitations of these factors in special adult circumstances and in the pediatric age group, respectively. Recent publications by the Work Group of the National Kidney Foundation (3) also reaffirm the limitation of basing GFR only on serum creatinine. Levey et al. (4) reviewed data from 1628 patients enrolled in the Modification of Diet in Renal Disease Study (MDRD). Their stated purpose was "to develop an equation from MDRD Study data that could improve the prediction of GFR from serum creatinine". Of the 1628 patients selected, data from 1070 were used to derive the equations, and data from 558 were used to verify the equations. They concluded that GFR calculated from serum creatinine, albumin, urea, and basic patient demographic data closely approximates the GFR determined from direct methods such as renal clearance of [¹²⁵I]iodothalamate (4). Their prediction equations 6 and 7 decreased unexplained variance from measured GFR by more than one-half when compared

with measured creatinine or urea clearance or the Cockcroft–Gault equation, i.e., from 19.6% to a range of 8.8–9.7%.

I would appreciate the authors' comments regarding the calculated GFR as opposed to use of a separate, additional serum analyte such as cystatin C. The calculated GFR is based on widely available and relatively inexpensive serum analytes and requires a single blood specimen. With the ubiquitous use of computers in the clinical chemistry laboratory, no human intervention for calculation is required for reporting relative to an individual test result. Consequently, the incremental cost relative to measuring serum creatinine by itself is less than US \$1.00. Perhaps the authors have data for calculation and comparison of this approach to GFR with the proposed cystatin C or other low-molecular weight analytes that they have studied.

We now provide our medical staff with an orderable calculated GFR based on equation 7 from Levey et al. (4). This closely approximates equation 6, but does not require a urine specimen. We also include the calculated GFR in our basic metabolic chemistry panel because the analytes required for its calculation are already being measured.

The automated measurement of serum albumin, creatinine, and urea with the aid of computerized calculations and reporting will most likely make the calculated GFR the standard of care for evaluating renal function. Solo measurement of serum creatinine for this purpose may become an anachronism.

References

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