Letter to the Editor

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Pre-analytical stability of 25(OH)-vitamin D in primary collection tubes

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To the Editor,

Globally, an upsurge is experienced in awareness and original research on vitamin D status. Pre-analytic stability and storage variables play an important part in test accuracy and repeatability, which have important implications for research, clinical practice and subsequent long-term storage in the clinical laboratory. Guidelines on specimen preservation and practice are available. However, very little specific information is provided on storage of serum specimens in primary blood collection tubes compared with secondary preservative-free plastic tubes. Anomalous 25(OH)-vitamin D results have been documented when serum was stored in primary gel-containing tubes with certain methodologies [1]. Storage of specimens in the primary tube may have several advantages for the laboratory, including decreased patient identification error, reduction in aliquoting potentially biohazardous samples, cost saving in materials and labour.

In our setting, we use 5 mL Becton Dickinson (BD) Vacutainer serum separator tubes (SST) (Becton Dickinson; Woodmead, South Africa) to collect blood for 25(OH)-vitamin D total analysis. These tubes contain a separation gel that forms a physical barrier between serum and blood cells during centrifugation. The manufacturer does not recommend the storage of specimens in primary gel separator tubes. The aim of our study was to investigate the possible impact of such storage on results over time.

The Ethics Committee of the Faculty of Health Sciences, University of the Free State in Bloemfontein, South Africa, approved the research protocol (ECUFS 162/2012).

We conducted a stability study of serum 25(OH)-vitamin D total using 19 specimens, which were collected as part of a larger study on vitamin D levels in type 2 diabetic patients. These study subjects did not receive any vitamin D supplementation. Specimens were collected into 5 mL BD SST tubes containing a serum separator gel. We examined the effect of storage on the 25(OH)-vitamin D concentrations in serum stored at –20 °C in the primary SST tube to that of a tube with no additives. The specimens were centrifuged immediately after receipt in the laboratory. Both the serum in the primary SST tube and a serum aliquot in an additive-free tube were stored at –20 °C.

Analysis of 25(OH)-vitamin D total level was done in a single run on a Cobas E601 analyser (Roche Diagnostics GmbH, Mannheim, Germany) with the manufacturer-recommended vitamin D3 (25-hydroxy vitamin D) immunoassay. Roche's package insert claims specimen stability at –20 °C for up to 24 weeks. Time in storage ranged from 21 to 144 days, with a mean of 73 days. 25(OH)-vitamin D concentrations in the clean container ranged from 11.44 to 108.4 nmol/L [mean 53 nmol/L, standard deviation (SD) 28.0 nmol/L] and 10.57–103.3 nmol/L (mean 51 nmol/L, SD 28.3 nmol/L) for storage in the primary tube. The 25(OH)-vitamin D concentrations were distributed across the entire range, including samples classified as vitamin D sufficient (>75 nmol/L), insufficient (50–75 nmol/L) and deficient (<50 nmol/L) [2], with the majority of our samples in the insufficient and deficient range.

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Data were analysed by performing a standard regression analysis in Microsoft Excel. As shown in Figure 1, we found a good linear (r=0.99) correlation between 25(OH)-vitamin D concentrations for SST-stored serum compared with serum stored in additive-free tubes. The distribution of the differences between the methods demonstrates a slight negative bias of –1.5 nmol/L [95% confidence interval (CI) –5.0; 2.1] and slope 1.0 (95% CI 0.9; 1.1).

Vitamin D is stable under variable pre-analytical conditions, including exposure to multiple freeze-thaw cycles [2]. Stability at room temperature was also demonstrated following storage in a primary separating gel tube (SST) for up to 7 days, and at –80 °C both in primary (SST) tubes and non-additive tubes for up to 12 months [3, 4]. Even though the polyester-based gel is inert [5], adsorption of analytes, such as therapeutic drugs (tricyclic antidepressants), tumour markers (CA-125) and steroid hormones (estradiol, progesterone), can lead to inaccurate analyte concentrations in cases of prolonged storage [5]. Whilst high precision liquid chromatography (HPLC) yielded variable 25(OH)-vitamin D levels for serum stored in SST gel tubes, no evidence exists of such possible interference with an immunoassay method [6]. Although Becton Dickinson recommends that serum should be separated and not stored in the primary gel tube [5], we found a good correlation in 25(OH)-vitamin D total stored at –20 °C in primary SST tubes and in clean plastic tubes. The specimens stored frozen on gel yielded a statistically and clinically non-significant lower result. The slight bias was not explained by time period stored on the gel (Figure 1). These findings are similar to those reported by Wielders [2] and Mathew [3], which suggest that 25(OH)-vitamin D is more stable than initially thought.

Despite the Clinical and Laboratory Standards Institute (CLSI) recommendation to analyse 40 samples spanning the test method range [7], only 19 samples were included in our stability study due to limited funding. 25(OH)-vitamin D total, not the isoforms 25(OH)-vitamin D_2 and D_3, was determined and evaluated for stability. Possible rapid binding of 25(OH)-vitamin D to the gel prior to centrifugation and aliquoting could cause a falsely decreased value. This is expected to affect both samples equally as this is a likely function of the pre-analytical instability prior to frozen storage. This would also affect all routinely collected vitamin D samples similarly to those selected for long-term storage. The stability of vitamin D samples was only evaluated for immunoassays and not for other methodology including HPLC. The influence of a decreased serum-to-gel ratio can be expected to affect sample stability and possible binding to the gel. However, in our study no low volume samples, including paediatric or under-filled samples, were evaluated. Only SST tubes were evaluated at –20 °C storage conditions, as this is our current laboratory practice. Alternative collection tubes including plasma separation tubes and products from other manufacturers were not evaluated. Further to this stability at varying storage temperatures, including room temperature, storage at 2–8 °C or –80 °C was not investigated. It would be a sound approach to consider this as a preliminary study with a view to include a larger sample size and different automated analysers in future. We found that duration of storage did not influence the

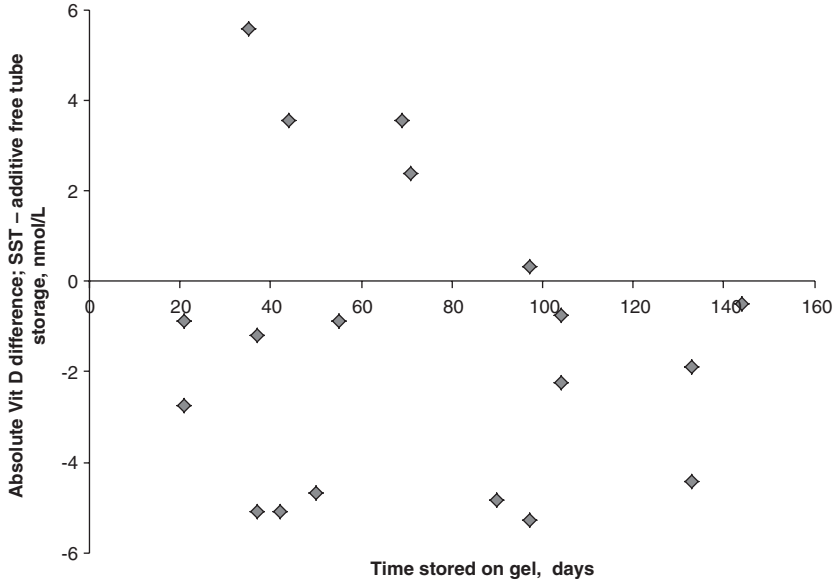

Figure 1 Bland-Altman graph demonstrating the difference of 25(OH)-vitamin D level in different storage tubes over time.

analyte concentration. Should this study be expanded, variation may be reduced by using pre-specified storage time points for vitamin D analysis.

Our study sample of 19 was small, which posed some minor limitations. Nevertheless, we conclude that serum for 25(OH)-vitamin D total analysis with immunoassay method can be stored in the primary gel separator tube, SST at –20 °C for up to 144 days without significant change. This finding may also prove useful when selecting and storing samples for additional research or retrospective research studies.

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