Short Report

Elevated results in a vitamin B_{12} assay when using serum separator blood collection tubes

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Abstract

Background In the light of apparently spurious serum vitamin B_{12} results in some patients, the effect of serum separator sample tubes on serum vitamin B_{12} values, assayed by the Bayer Centaur analyser, was examined.

Method Results of parallel assays of serum vitamin B_{12} in plain (non-gel) serum tubes and serum separator gel tubes were compared. Serum in previously centrifuged gel tubes was mixed in the tube and the effect of that mixing on assay results quantitated. A limited investigation of the effect of tube cap type was also carried out.

Results Serum vitamin B_{12} concentration was 54% higher in samples taken into serum separator tubes after re-mixing the serum in the original tube. This effect could be abolished by re-centrifugation.

Conclusions Use of serum separator tubes may be associated with spuriously elevated serum vitamin B_{12} concentrations in the Bayer Centaur assay. Laboratories receiving samples for vitamin B_{12} assay in serum separator tubes that have already been centrifuged should either re-centrifuge the tubes, or aliquot and re-centrifuge serum from the tubes prior to vitamin B_{12} assay.

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Introduction

Requests for the measurement of vitamin B_{12} are common in clinical laboratories. Our laboratory serves population locations throughout New Zealand. We receive, on average, 140 requests for serum vitamin B_{12} assay daily. Because of the geographical spread of the population served, some of the samples we receive are taken into serum separator tubes (SSTs) and centrifuged at or near the point of collection. Thus, samples are transported to and received at the laboratory already centrifuged with the serum on the separator gel. General concern about possible effects of the separator gel on some laboratory assays has been expressed for some time, but most attention has focused on therapeutic drug monitoring.[1,2] A recent report demonstrated an effect of these tube types on tri-iodothyronine assays.[3] We decided to investigate the delay and transport aspects of our pre-analytical sample handling processes and investigate the effect of the use of SSTs on vitamin B_{12} measurements.

Method

Thirty-three consecutive patients from whom samples had been taken into a plain glass serum tube (PT) and a glass SST for routine biochemical testing were identified. Testing was at the instigation of a general practitioner and included a request for serum vitamin B_{12}. All blood collection tubes used were manufactured by Becton Dickinson (Mount Wellington, Auckland, New Zealand) and were used within their expiry date. All SSTs used had a 9.5-mL capacity (item number 366510), except for three that had a 4-mL capacity (item number 367695). All the PTs used had a 7-mL capacity (item number 367694).

Samples were allowed to clot for 30 min, after which they were centrifuged at 2750 *g* for 10 min. Vitamin B_{12} was assayed in both the PT and SST serum samples using the primary tubes. The centrifuged SSTs were then mixed on a platform mixer for 150 min at room temperature to simulate the effect of sample mixing

during transport. Vitamin B_{12} was re-assayed in this SST sample before and after re-centrifugation.

An additional ten samples were used to investigate any effect of the tube cap. After centrifugation of samples in PTs, serum was aspirated from the clot, placed into an unused PT (item number 366430) and vitamin B_{12} was assayed. The serum was then mixed for 150 min in the same tube and vitamin B_{12} re-assayed, after which the original cap was replaced with one from an unused SST (item number 366510). The serum was re-mixed for 150 min with the new cap in place then re-assayed.

Serum vitamin B_{12} was assayed using the Bayer Centaur chemiluminescence method (Bayer Diagnostics, Glenfield, Auckland, New Zealand). The assay was subject to satisfactory internal and external quality control during the study. Between-batch coefficients of variation were 6.8% at 228 pmol/L, 7.7% at 472 pmol/L and 4.3% at 872 pmol/L. Comparison of group medians was carried out using the Wilcoxon matched-pairs signed-ranks test.

Results

Unless stated otherwise, results are expressed as group median (inter-quartile range). Serum vitamin B_{12} concentration in the PT [240 (109) pmol/L] was significantly different from that in the SST [256 (138) pmol/L; $P = 0.001$]. Mixing the serum in the centrifuged SST caused an increase in the median serum vitamin B_{12} concentration to 394 (157) pmol/L ($P < 0.0001$). This rise in concentration was all but abolished by re-centrifugation of the sample, which resulted in a serum vitamin B_{12} concentration of 249 (137) pmol/L (*see* Fig. 1). This value was significantly different from the initial value in the SST (difference 12.5 pmol/L, 95% confidence interval 4.5–20.5, $P = 0.003$). In three samples that were taken into 4-mL SSTs, the relative increases in vitamin B_{12} concentration after the mixing step were 111%, 56% and 96%. This increase was removed by centrifugation.

Mixing serum in a PT for 150 min with an SST cap resulted in a median serum vitamin B_{12} concentration of 270 (83) pmol/L, which was not different from that in the original PT [283 (95), $P = 0.19$].

Discussion

These results indicate that assay of vitamin B_{12} in blood collected into Becton Dickinson SSTs and transported from distant locations, without any further sample preparation, is likely to result in spuriously elevated values when using the Bayer Centaur assay. There appears to be an interferant in the SST that can be almost completely removed by re-centrifuging the sample. Our admittedly limited studies suggest that the interference effect is not likely to be due to the tube cap or materials applied to it.

This study could be criticized for the way in which the effect of transport was modelled. Continuous mixing for 150 min probably causes more disturbance of the gel barrier than may occur in routine transport of samples to the laboratory from distant locations. However, we feel that this is a standardized procedure that can be replicated in other laboratories and for other assays. Separately, we have demonstrated that transport of SSTs in a company courier van for 24 h results in a marked rise in serum vitamin B_{12}. This interference is removed by centrifugation (unpublished data).

The Bayer serum vitamin B_{12} assay is a competitive assay in which labelled B_{12} competes with vitamin B_{12} in serum to bind to intrinsic factor covalently coupled to magnetic particles. The apparent increased recovery suggests that there is an apparent reduction in the binding of labelled vitamin B_{12} to the solid phase. This may be because the labelled B_{12} is bound by the interferant, preventing it binding in turn to the solid phase, or that the interferant itself binds to the solid phase, thus preventing the binding of label. A further possibility is that the interferant inhibits generation of the chemiluminescent signal. Finally, it is possible that initial centrifugation fails to completely remove cellular and particulate matter and that this material remains on the surface of the gel. It then becomes mixed in the serum during transport and

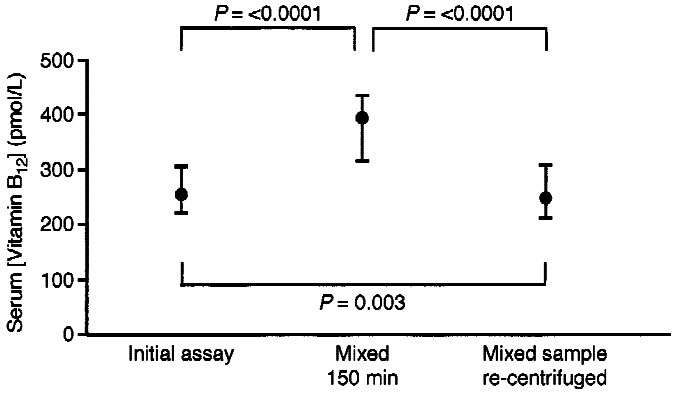

Figure 1. *Serum vitamin B_{12} concentration in serum separator tubes immediately after initial centrifugation, after mixing for 150 min and after re-centrifugation. Solid circles indicate the median, bars the 98% confidence interval.* P *values indicate the level of statistical significance for the differences between the medians of the groups indicated.*

interferes in the assay if re-centrifugation is not carried out. Whichever is the case, it appears to be a loose binding in that centrifugation removes the interference.

The small but statistically significant differences seen in vitamin B_{12} values in the SST after the second centrifugation step compared with that on initial analysis may be attributable to sample degradation. Vitamin B_{12} is susceptible to degradation as a result of exposure to light and higher temperatures. As there were approximately 3 h between the assays, it is possible that some sample degradation may have occurred. The difference between the initial PT and SST values is most likely to be attributable to the same interferant that caused the marked rise in values after mixing.

The additional centrifugation step required to remove this interference is likely to cause disruption of the flow of work through the laboratory and, if the assay is carried out on the original blood collection tube, is likely to lead to artifactual changes in other serum analytes (e.g. potassium).[4] It may be preferable, therefore, to centrifuge an aliquot of serum taken from the SST prior to analysis.

The effect described is another indication that laboratories need to be aware of all aspects of the pre-analytical process that impact on generation of clinically relevant and accurate results. Continued good assay performance, as judged by analysis of internal and external quality assurance samples, will not reveal the sort of problem described here and laboratory staff should continue to be vigilant.

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