highlighted by the insert presented in Fig. 1A. The Bland-Altman plot (Fig. 1B) confirmed this random tendency, which was independent of concentration and of sex, and also demonstrated an amplified dispersion at 25-OHD concentrations >50 nmol/L.

Our data (Fig. 1B) suggested that CLIA results tended to be higher than RIA at low and high concentrations, conversely to data previously published (4). With a threshold of 25-OHD ≥30 nmol/L but <50 nmol/L to define vitamin D insufficiency, in 33% of the 54 individuals with 25-OHD concentrations classified as insufficient by RIA, 25-OHD concentrations were normalized by CLIA (range: 53.0–109.5 nmol/L). In contrast, 21% of the 62 study participants with 25-OHD concentrations ≥50 nmol/L by RIA had insufficient 25-OHD concentrations by CLIA (range: 17.5–48.0 nmol/L). For optimal serum 25-OHD concentration defined as >75 nmol/L in osteoporotic patients, 35% of the 23 patents with 25-OHD concentrations above this threshold by RIA had concentrations below it by CLIA. The RIA method used a primary antibody to 25-OHD in a homogenous phase with a 2nd antibody used as precipitating agent, whereas CLIA used the same primary antibody immobilized onto coated magnetic particles. This antibody interacts differently with the first calibrator [i.e., 17.5 nmol/L (CLIA); 12.5 nmol/L (RIA) with an optional calibrator of 6.25 nmol/L (B/B0: 91%) created by diluting 12.5 at 1:2 as suggested by the manufacturer], indicating different affinity profiles that are probably responsible for these random results. These divergent results may also be attributable to different calibrators, constituted in either human- (RIA) or horse-based serum (CLIA), different incubation times (90 min by RIA vs 30 min by CLIA), or an insufficient quantity of reagents used to dissociate 25-OHD from its binding protein.

Overall, these 2 methods did not similarly classify individuals with reference to well-known arbitrary cutoff values. The random tendency observed whatever the concentrations measured did not permit the definition of a clear strategy concerning patient follow-up, particularly for those needing treatment with respect to their vitamin D status. The discrepancy between these 2 methods is consistent with either important negative (4, 5) or positive (our data) intercepts traducing differences in the assay response to the calibrant matrix.

Finally, our results are consistent with the poor correlation previously reported between RIA and CLIA (5) and demonstrate that in disagreement with recently published data (4), a 25-OHD value <50 nmol/L used to define vitamin D insufficiency with the DiaSorin RIA is not suitable for use with the LIAISON® assay (CLIA).

Fidaa Ibrahim
Christine Parmentier
Philippe Boudou*

Unit of Hormonal Biology
Hôpital Saint-Louis
Assistance Publique-Hôpitaux de Paris
Paris, France

*Address correspondence to this author at: Unit of Hormonal Biology, Saint-Louis University Hospital (AP-HP) and INSERM U 671, 1 avenue Claude Vellefaux, 75475 Paris cedex 10, France. Fax 33-1-42-49-42-80; e-mail: philippe.boudou@ls.aphp.fr.

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References


High Total Protein Impairs Appropriate Gel Barrier Formation in BD Vacutainer Blood Collection Tubes

To the Editor:

Many laboratories perform routine chemistry analysis with serum or plasma–based blood collection tubes containing separator gels. A barrier polymer is present at the bottom of the tube. The density of the material causes it to move upwards during centrifugation to the supernatant–cell interface, where it forms a barrier separating plasma or serum from cells. Supernatant plasma or serum may be aspirated directly from the collection tube, eliminating the need for transfer to a secondary tube.

We recently observed 2 occasions within 1 month when both the insensitive electrode and chemistry sampling probes of the analyzer (Modular Analytics, Roche Diagnostics) were occluded. In both cases, the occlusion was caused by inappropriate gel barrier formation after centrifugation (200g for 10 min at room temperature) of the primary tubes. Plasma (BD Vacutainer® PST™ II) and serum (BD Vacutainer® SST™ II) samples had been collected from 2 patients diagnosed with multiple myeloma. In the plasma tube, the gel barrier material was floating on the surface of the supernatant, and in the serum tube the gel barrier was entwined with the serum and erythrocytes (Fig. 1). Analysis of blood samples from both patients in plain serum tubes showed highly increased total protein concentrations (139 and 142 g/L; reference interval 60–80 g/L) caused by the presence of a monoclonal-protein (an IgG-κ of 89 g/L and an IgA-κ of 92 g/L, respectively). Furthermore, plasma viscosity values were 5.7 and 7.1 centipoise, respectively, (reference interval 1.5–2.0 centipoise) and specific gravities, as measured by weighing 500 μL of plasma or serum, were 1.037 and 1.039, respectively.

The positioning of the gel in the tube is influenced by a number of variables, some of which are con-
trolled by the tube manufacturer (specific gravity, yield stress, viscosity, density, and tube material), some by the hospital laboratory (centrifugation speed, temperature, acceleration and deceleration conditions, and storage conditions), and some of which are patient specific [heparin therapy, low hematocrits, increased plasma proteins (1)], and the use of iodinated blood contrast media (2)].

A retrospective analysis in our clinical chemistry laboratory of total protein requests showed that during a 5.5-year period, 5 of 13,221 patients (0.04%) had a total protein concentration $\geq 110$ g/L. Therefore, we anticipate that our laboratory should observe this phenomenon several times a year. This number may vary depending on the degree of oncology-related patients visiting the hospital. To our knowledge, only one single case report has been published on this topic for a blood collection system from a different manufacturer (1).

Laboratories, in which preanalytical steps include automatic centrifugation and sample transport to on-line chemistry analyzers, are particularly vulnerable for occlusion of sample probes from inappropriately separated blood samples. Visual checks to determine the adequacy of barrier formation after centrifugation should prevent the inappropriately separated samples from being transferred to the analyzer, although labels on tubes can often prevent rapid visual inspection.

Despite the fact that inappropriate barrier formation is occurring at a low frequency, the impact on costs (sample probe replacement and downtime of the analyzer causing discontinuation of the workflow process) and patient outcomes (e.g., potential danger of reporting falsely low results when no sample is aspirated) can be substantial. Laboratories and tube manufacturers should be aware of the limitation of using any tubes containing gel-separator in patients with high plasma viscosity because of the presence of high total protein concentrations. In these particular cases, subsequent blood drawings should be collected in non–separator-based blood collection tubes. We will conduct further with the tube manufacturer to assess the amount of total protein at which gel barrier formation is compromised. Our observation contributes to the increasing awareness of the impact on patient outcomes and the costs of laboratory errors occurring in the preanalytical phase (3).

Fig. 1. Gel barrier formation in plasma (A, B) and serum (C) separator tubes in patient 1.

References

Johannes M.W. van den Ouweland1*
Stephan Church2
1 Canisius-Wilhelmina Medical Centre
Department of Clinical Chemistry
Nijmegen, The Netherlands
2 BD Diagnostics
Preanalytical Systems
Plymouth, United Kingdom

*Address correspondence to this author at: Canisius-Wilhelmina Medical Centre, Department of Clinical Chemistry, Weg door Jonkerbos 100, 6500 GS Nijmegen, The Netherlands. Fax 31-24-3658671; e-mail j.v.d.ouweland@cwz.nl.
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Survival Related to Plasma C-Reactive-Protein in Nonagenarians Is Modified by Apolipoprotein E Genotype

To the Editor:
In the present study, we combined 2 thoroughly studied markers of inflammation and lipid metabolism—sensitive C-reactive protein (hsCRP) and apolipoprotein E genotype (apoE)—to predict mortality in the elderly (1, 2). Plasma hsCRP concentration (1) and apoE genotype (2) are both important predictors of coronary artery disease (CAD) and stroke (1, 2). The risk of myocardial infarction in people with hsCRP concentrations in the highest third of the population range is twice that of those with hsCRP in the lowest third (1). ApoE genotype is a key regulator of lipoprotein metabolisms (2, 3), and the e4-allele has been associated with in-