**Anomalous Flotation of Separator Gel: Density or Viscosity?**

**To the Editor:**

Blood collection tubes containing a thixotropic polyester gel are widely used for clinical laboratory testing. The gel, because of its intermediate density (1.04 g/cm³), physically separates the liquid component of the blood from the cells (densities of 1.03 and 1.09 g/cm³ respectively) after centrifugation, and forms a barrier between the liquid phase and the cells. However, recent cases have been reported in which anomalies in the formation of this separating barrier can occur (1). Although the number of reported cases seems low, the impact on cost and the risk of reporting misleading values is substantial (1). Different interpretations have been proposed regarding whether the anomalous flotation of the gel is a matter of density or viscosity of the serum/plasma (2–4). We investigated whether this gel behavior was due to the high density or to the high viscosity of the liquid phase in a case of floating separator gel in samples from a 70-year-old woman in whom IgA λ myeloma was subsequently diagnosed.

Blood samples were received in BD Vacutainer® PST™ II tubes that were processed in the routine manner. After centrifugation (1200g for 10 min) the gel was observed floating on the supernatant instead of separating the plasma from the cells. Interestingly, when BD Vacutainer SST™ II tubes were used, gel barrier formation was correct and there were no inconsistencies in the serum/clot separation. Significant laboratory data from serum included: Ca 126 mg/L, hemoglobin 81 g/L, hematocrit 24.8%, total protein 119 g/L, erythrocyte sedimentation rate 165 mm/h. Ig quantification by nephelometry showed an IgA concentration of 102 g/L, which was the highest concentration ever observed in our laboratory.

These data suggested that the floating-gel phenomenon in plasma (1) was attributable to high total protein concentrations, which are associated with increases in both density and viscosity, whereas the lack of fibrinogen in serum decreased the density and/or viscosity, resulting in the correct formation of the gel barrier in the serum-separator tubes.

To investigate whether the increased density or the viscosity of the plasma was responsible for the anomalous gel barrier formation, we performed experiments to simulate conditions with density and viscosity values (1.039 g/cm³ and 7.1 mPa·s, respectively) found in plasma in a case (1) with protein and Ig content quite similar to ours.

We prepared solutions of dextran in solution with normal saline as solvent. Densities and viscosities were measured with an Anton Paar Density Meter-DMA 35 and an Ubbelohde-viscometer, respectively. Samples were collected into PST II tubes and centrifuged at 1200g for 10 min. Results are shown in Table 1.

Our results demonstrate that, irrespective of the high viscosity values (reference interval 1.5–2.0 mPa·s), the gel position is determined only by the density of the solution. Even in the case of the 6% dextran solution, which had a density very close to that of the gel, it was not necessary to extend the centrifugation time to get the right gel position.

In summary, our results confirm that tubes containing gel separator may fail to form an appropriate barrier in blood samples with high protein content. This anomalous behavior is likely attributable to the high density of plasma/serum, because dextran solutions with very high viscosity but normal density allow proper behavior of the separator gel. These findings suggest that any substance in blood that increases density substantially can give rise to anomalous flotation of separator gel. The information provided by this study may lead to improvements in the characteristics of these gels and determination of the amount of protein that hinders appropriate gel barrier formation (1).

**Table 1. Density, viscosity and position of the gel in dextran solutions and normal samples.**

<table>
<thead>
<tr>
<th>Dextran, %</th>
<th>Density, g/cm³</th>
<th>Viscosity, mPa·s</th>
<th>Position of the gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1.051</td>
<td>8.61</td>
<td>Floating</td>
</tr>
<tr>
<td>8</td>
<td>1.045</td>
<td>7.28</td>
<td>Floating</td>
</tr>
<tr>
<td>6</td>
<td>1.038</td>
<td>5.74</td>
<td>Nonfloating</td>
</tr>
<tr>
<td>Normal serum</td>
<td>1.023</td>
<td>1.60</td>
<td>Nonfloating</td>
</tr>
<tr>
<td>Normal plasma</td>
<td>1.025</td>
<td>1.80</td>
<td>Nonfloating</td>
</tr>
</tbody>
</table>

**Grant/Funding Support:** None declared.

**Financial Disclosures:** None declared.

**References**

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**Impact of Blood Sampling on the Circulating Matrix Metalloproteinases 1, 2, 3, 7, 8, and 9**

**To the Editor:**

Matrix metalloproteinases (MMP) play a crucial role in numerous pathological processes. Because cellular changes may be reflected in body fluids, measurements of MMP in blood have been recommended as noninvasive tools in diagnosis and monitoring of diseases (1). MMP measurements can be affected by the blood sampling procedures, as shown for gelatinase B (EC 3.4.24.25; MMP-9), for which higher concentrations have been noted in serum than in plasma samples (2, 3). However, the impact of the blood sampling process on measurements of many other MMPs has not yet been studied in detail.

Our aim was to demonstrate the effect of blood sampling on the concentrations of collagenase-1 (EC 3.4.24.7; MMP-1), stromelysin-1 (EC 3.4.24.17; MMP-3), matrilysin (EC 3.4.24.33; MMP-7), and collagenase-2 (EC 3.4.24.34; MMP-8) circulating in blood. Because the preanalytical impact of blood sampling is well known for gelatinase A (EC 3.4.24.24; MMP-2) and MMP-9 (4), we measured these substances for use as comparisons. The study was performed in accordance with the ethical standards of the Helsinki Declaration and was approved by the ethical board of the hospital. All sample donors recruited on a voluntary basis and informed about the objectives of the study provided written informed consent.

During single venipunctures performed in 10 healthy adults (4 female, 6 male; mean (SD) age, 42 (14) years) venous blood samples were collected in plastic tubes (Sarstedt) without additives or with kaolin-coated granulate as coagulation accelerator (serum(−)) and serum(+) respectively) and in tubes coated with lithium heparin, disodium citrate, or dipotassium EDTA in that specified sequence and centrifuged within 30 min after venipuncture (1600g; 15 min; 4 °C). The supernatants were removed and stored at −80 °C until analysis. MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-12, and MMP-13 were simultaneously measured using the Fluo- or kinase MultiAnalyte Profiling assays (R&D Systems) on a Luminex 100 Bioanalyzer (Luminex,). The assays measure pro-, mature, and tissue inhibitor of metalloproteinase (TIMP)-1–complexed MMPs with <0.5% cross-reactivity between the MMPs.

Our data demonstrate that in blood the measured concentrations of not only the gelatinases but also of other MMPs are strongly influenced by the sampling procedure. Our study is the first detailed investigation of simultaneous measurements of multiple MMPs in serum with and without clot activator, and in citrate-, EDTA-, or heparin-plasma. Our findings highlight the

**References**


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**Letters**

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important differences between the MMP concentrations in serum collected with and without clot activator as well as the differences among both serum types and plasma samples. It is noteworthy that MMPs such as MMP-1, MMP-8, and MMP-9 are abundantly expressed in platelets and leukocytes. Increased MMP concentrations in serum may be due both to their release during coagulation and to their secretion, which is induced by the clot activator itself, as has been shown for MMP-9 (3, 4).

In conclusion, each MMP to be analyzed as a circulating biomarker in blood requires careful consideration of the preanalytical effects of blood collection methods. Despite the currently available knowledge base, some studies performed on gelatinases and collagenases in serum do not address the concerns raised by reports of pitfalls and misinterpretations linked to preanalytical aspects of blood processing. Our results indicate that the use of serum should be avoided for measurement of MMP-1, MMP-8, and MMP-9. In contrast, MMP-2, MMP-3, and MMP-7 measurements showed comparable values among citrate- and heparin-plasma and the serum samples, with citrate-plasma seems to be the sample of choice for the measurement of all MMPs.

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References

To the Editor:

In the January 2008 issue of Clinical Chemistry, Lum et al. (1) reported on their recent experience with the Multigent benzodiazepines assay. We thank Dr. Lum and his colleagues for their comments regarding the assay, and value their insights and expertise in this area. Consistent results from a screening assay that detects multiple compounds belonging to a specific drug class requires an analytical balancing act, because the reagent performance must minimize false negatives while also maximizing confirmation rate by various confirmatory methods. As the manufacturer of this assay, we recognize that this process leads to issues that may concern users of our products.

The Multigent benzodiazepines assay is marketed as a screening assay, and the package insert states that a more specific alternate chemical method must be used to confirm the screening result. This specific Multigent reagent uses polyclonal antibodies, which inherently are subject to changes in specificity and sensitivity. When it became necessary to change antibody preparations, a customer bulletin was sent to users of this product in May 2007 to notify them of this change. The customer bulletin also suggested that a user’s confirmatory method must be able to detect benzodiazepines at concentrations lower than those detected by the Multigent assay.

We are not entirely clear regarding the definition of “false positive” being employed in Dr. Lum’s laboratory. A false positive may be defined in relation to a specific cut-off concentration, in which case detectable compounds may be present, but 2 methods may differ in their limits of detection for these compounds. Alternatively, a false positive may be defined as a result indicating that a drug is present even when a confirmatory method with a lower limit of detection for all compounds present indicates the compounds in question are undetectable. Although we do not dispute Dr. Lum’s findings, we are unsure if some detectable benzodiazepine drugs and related metabolites were observed to be absent by a confirmatory method but might be detected by the Multigent assay. We respectfully suggest the possibility that the Multigent assay might be responding to benzodiazepine-related compounds that were not included in the confirmation, or were not detected with sufficient limits of detection by the confirmatory analysis.

We realize that the antibody pool introduced in early 2007 performed differently from the antibody pool that had been previously used by Dr. Lum’s laboratory and acknowledge the need to return to the earlier performance characteristics. We have initiated a project to reformulate the Multigent benzodiazepines reagent to favor maximum confirmation rates, and we will notify users as soon as the reformulated assay becomes available.

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