adapter to fit regular needles and evacuated blood collection tubes. The design of the BD needle holder enables direct linear flow of blood from the venipuncture site into the blood collection tubes. The Greiner Holdex was designed with an offset luer adapter to enable a convenient puncture angle. Therefore, the straight path within the Holdex is interrupted twice by angles of 90°, thus forming three consecutive flow segments. These changes in the linear flow of blood might impose mechanical strain on blood cells, affecting membrane integrity, which may cause efflux of intracellular constituents into the serum.

We compared the Greiner system to the BD system by evaluating within-subject variations in the results of blood analyses. Fifty-five healthy individuals participated in our study (mean age ± SD, 26.7 ± 4.3 years). Seventeen volunteers were women (age, 25.8 ± 2.1 years) and 38 were men (age, 27.2 ± 5.0 years). All residents in Jerusalem and its environs. The study protocol was approved by the institutional research ethics board, and all subjects gave their informed consent.

The study participants were seated for 5 min before the tourniquet was applied. Hand-clenching was avoided. A 21-gauge needle attached to its specific holder (BD or Greiner) was inserted into the antecubital vein, and blood was collected into evacuated tubes (into the serum separator tube, followed by the citrate tube, and then into the EDTA tube). The tourniquet was released when blood began flowing into the first tube. The tubes were inverted six times after withdrawal from the holder to ensure proper mixing of the blood with anticoagulants. Phlebotomy was performed twice in each patient. By random assignment, the BD blood-collection system was used in one arm and the Greiner blood collection system was used in the opposite arm.

Serum and plasma were prepared by centrifugation of blood (1200g for 10 min) at room temperature within 30 min of collection. Each serum sample was analyzed for sodium and potassium using ion-selective electrodes (Cobas Integra chemistry analyzer; Hoffmann-La Roche). Additionally, aspartate aminotransferase, alanine aminotransferase, amylase, alkaline phosphatase, lactate dehydrogenase, bilirubin, HDL-cholesterol, magnesium (Mg²⁺), and calcium were determined by specific colorimetric assays (Cobas Integra analyzer). Differential blood counts were carried out using the Vega blood cell counter (ABX Hematology). Coagulation assays [prothrombin time (PT), activated partial thromboplastin time, and fibrinogen] were performed with the ACL 1000 coagulation analyzer (Instrumentation Laboratory). Hemoglobin in plasma was measured by spectrophotometric scanning technique as described previously (4).

Statistical analyses were performed with the use of SPSS statistical software (Ver. 7.5.21). Differences in quantitative variables were assessed for statistical significance with the Student paired t-test.

In blood samples obtained by the nonlinear blood collection system (Greiner), significant increases were observed in serum Mg²⁺ concentration, plasma hemoglobin, and PT (Table 1). All other analytes were essentially unchanged compared with the direct-flow system (BD). The 31% increase in hemoglobin was not accompanied by changes in serum potassium concentrations and lactate dehydrogenase activity. This indicates that the extent of erythrocytosis was relatively low and the leakage of intracellular constituents

<table>
<thead>
<tr>
<th>Analyte</th>
<th>BD</th>
<th>Greiner</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin in plasma, g/L</td>
<td>0.16 ± 0.16</td>
<td>0.25 ± 0.21</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>Mg²⁺, mmol/L</td>
<td>0.83 ± 0.057</td>
<td>0.85 ± 0.054</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PT, s</td>
<td>11.86 ± 0.9</td>
<td>12.86 ± 3.2</td>
<td>&lt;0.030</td>
</tr>
</tbody>
</table>

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References

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Effects of Blood-Collection Systems and Tubes on Hematologic, Chemical, and Coagulation Tests and on Plasma Hemoglobin

To the Editor:
Numerous preanalytical variables may affect the outcome of clinical laboratory tests (1–3). Blood-collection procedures are considered an important impact factor because they are associated with several possible sampling problems, including the use of a tourniquet with hand-clenching, the site of venipuncture, hemolysis because of venipuncture, clenching, the site of venipuncture, and blood began flowing into the first vein, and blood was collected into evacuated tubes (into the serum separator tube, followed by the citrate tube, and then into the EDTA tube). The tourniquet was released when blood began flowing into the first tube. The tubes were inverted six times after withdrawal from the holder to ensure proper mixing of the blood with anticoagulants. Phlebotomy was performed twice in each patient. By random assignment, the BD blood-collection system was used in one arm and the Greiner blood collection system was used in the opposite arm.

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Statistical analyses were performed with the use of SPSS statistical software (Ver. 7.5.21). Differences in quantitative variables were assessed for statistical significance with the Student paired t-test. In blood samples obtained by the nonlinear blood collection system (Greiner), significant increases were observed in serum Mg²⁺ concentration, plasma hemoglobin, and PT (Table 1). All other analytes were essentially unchanged compared with the direct-flow system (BD). The 31% increase in hemoglobin was not accompanied by changes in serum potassium concentrations and lactate dehydrogenase activity. This indicates that the extent of erythrocytosis was relatively low and the leakage of intracellular constituents
was negligible. Our results are consistent with the study by Sonntag (2), which showed that the concentrations of potassium and lactate dehydrogenase in serum are affected by hemoglobin concentrations ≥0.2 g/L.

Unexpectedly, we measured a 2.4% increase in Mg$^{2+}$ concentrations in the Greiner samples. We assumed that optical interference of hemoglobin caused the artifactual increase in Mg$^{2+}$ in these samples. Hypomagnesemia is associated with alcoholism, pancreatitis, gastrointestinal diseases, glomerulonephritis, hyperthyroidism, hyperparathyroidism, metabolic acidosis, and drug administration. Furthermore, in patients with acute myocardial infarctions, serum Mg$^{2+}$ <0.82 mmol/L may increase the risk of ventricular arrhythmia. Falsey increased concentrations of Mg$^{2+}$ might then be a concern if the Greiner system is used. Presumably, in such cases the Mg$^{2+}$ assay with an ion-selective electrode is preferable to the spectrophotometric method.

The 8% increase in PT values after blood collection with the Greiner system (Table 1) may be a result of interference with clot formation. We assume that membrane phospholipids exposed by the slight erythroctolysis may compete with the PT reagent (thromboplastin) used for the assay of extrinsic pathway factors, causing prolongation of PT. It is also possible that the different composition of the coagulation tubes (glass in the BD vs plastic in the Greiner) may have affected the results.

Although the degree of hemolysis was significantly higher in the Greiner system than in the BD system, all test results remained within the reference intervals for the young healthy individuals in our study. However, in hospitalized patients, quantitative differences in blood analytes may be accentuated, particularly when intravascular erythrocyte destruction is expected to occur, such as in bacterial or viral infections (5), hypersplenism, cardiac and hepatic abnormalities (6), exposure to venoms and toxins (6), and the use of oxidant drugs (7).

Obviously, the decision of which blood-collection system (or systems) should be used in a healthcare center depends on considerations of cost, safety, and convenience. Along with these considerations, it is appropriate to document the effect of a specific system on laboratory test results.

References


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Autoimmune Hypoglycemia Presenting as Seizure One Week after Surgery

To the Editor:

A 72-year-old Caucasian man was admitted to our hospital in November 1999 for reanastomosis of an ileostoma that had been created after perforation of a sigmoid diverticulum with peritonitis. The past medical history of the patient was unremarkable except for angioneurotic edema of unknown etiology 14 years previously. The immediate postoperative course was uneventful, and the patient received glucose (400 g/L, 40 mL/h) via a central line. Because it was planned to reestablish enteral nutrition on day 7, glucose infusions were discontinued during the preceding night. At 0500 on day 7, the patient had a generalized seizure. Bedside testing revealed a nondetectable blood glucose concentration. After the patient received 100 mL of a glucose solution (500 g/L), the seizures stopped. During the following 24 h, his glucose concentration repeatedly fell to <2.8 mmol/L, requiring glucose administration; 36 h after the initial hypoglycemia, the patient was on full enteral nutrition, and glucose concentrations remained stable.

A serum sample obtained 1 h after the seizure revealed an extremely high insulin concentration (1746 mIU/L; reference interval, 6–35 mIU/L; IMx Insulin; Abbott) and a serum glucose concentration of 4.3 mmol/L, whereas C-peptide was increased approximately twofold above the upper reference limit (8.8 μg/L; reference interval, 0.8–4 μg/L; Immulite C-Peptide; DPC). In subsequent samples, insulin concentrations were lower, but they were still 5–15-fold above the upper reference limit. A computed tomography, obtained preoperatively, did not reveal a pancreatic mass.

To exclude an analytical artifact of insulin determination, samples were reanalyzed in serial dilutions up to 1:32 and with a different assay (Count-a-coat Insulin; DPC); this confirmed extreme hyperinsulinemia. Serum insulin antibodies (RIA) were 88 kilounits/L (reference interval, 0–5). On the basis of these findings, autoimmune hypoglycemia (AIH) (1) was suspected.

To confirm the diagnosis, the patient was evaluated by an oral glucose challenge (75 g) after full recovery from surgery. Glucose intolerance was observed with a serum glucose of 13.0 mmol/L after 60 min; insulin was increased to a peak concentration of 3162 mIU/L after 180