Effect of Serum Volume and Time of Exposure to Gel Barrier Tubes on Results for Progesterone by Roche Diagnostics Elecsys 2010

To the Editor:

Inert thixotropic gel is commonly used in blood collection tubes for separation of serum or plasma from the cellular components of blood. Eichhorn et al. (1) in 1997 suggested that gel tubes (Sarstedt) used in their laboratory were generally reliable, but they found incomplete separation in 3.1% of tubes. Plain tubes are preferred for therapeutic drug monitoring because of the potential for binding of drugs to the gel (2). Smith (3) in 1985 and Hilborn and Krahn (4) in 1987 found that progesterone decreased with time as serum remained in contact with the separator gel. In contrast to these findings, we recently encountered an apparent increase in progesterone concentrations when blood was collected in glass serum separator tubes (SST® with Clot Activator; Becton Dickinson) and progesterone was measured by the Roche Diagnostics Elecsys® 2010.

To further investigate these findings, we evaluated the effect of SST on several assays performed on the Elecsys 2010. All test samples (treated in compliance with the Human Investigation Committee) were assayed for progesterone, estradiol, luteinizing hormone (LH), follicle-stimulating hormone, creatine kinase MB isoenzyme, and troponin T. Blood from three volunteers was collected in both SST (16 × 100 mm) and plain glass (13 × 100 mm) Vacutainer® tubes and was allowed to clot for 30 min before centrifugation. Serum drawn in SST remained on the separator gel, whereas serum drawn in plain tubes was transferred into 13 × 100 mm glass tubes. Samples were kept capped at room temperature and tested at 0, 2, and 4 h after collection; they were then refrigerated and tested at 24 h. In an additional progesterone study, serum volumes of 2, 5, and 10 mL were allowed to sit in SST, and progesterone was assayed at 0, 2, and 4 h.

Measured progesterone was markedly higher in the sera in SST tubes than in sera in plain tubes (Fig. 1, top panel). The effect of gel barrier on progesterone was greater when tubes contained smaller serum volumes (2 or 5 mL), and the effects increased with time (Fig. 1, bottom panel). There were no significant differences between SST and plain tube results for estradiol, LH, follicle-stimulating hormone, prolactin, creatine kinase MB isoenzyme, and troponin T. We also had access to blood samples collected simultaneously in plain and SST tubes from five patients on digoxin. The time from blood draw to testing for this group ranged from 6 to 8 h. These samples were assayed for digoxin, estradiol, progesterone, and prolactin. We found no clinically significant difference between plain and SST patient sample results for digoxin, estradiol, or prolactin, but the trend for progesterone results was similar to those in the top panel of Fig. 1, with progesterone results higher by 0.6–2.2 μg/L for the SST samples.

We conclude that Elecsys progesterone results are falsely increased when blood is collected in these SST containers and that a component of the inert gel is probably released into the serum to produce the false increase. It is difficult to conclude that this represents an interference with the electrochemiluminescent measurement because there was no similar interference for the other assays tested, and the mechanism is unknown. Although the absolute increases in serum progesterone values are small, without the support of estradiol and/or LH values, a pa-

Fig. 1. Effect of exposure time on serum progesterone results for SST and plain tubes in volunteers A, B, and C (top) and effects of serum volume and exposure time on progesterone results for SST and plain tubes in volunteers A and B (bottom).
tient’s menstrual cycle could be interpreted as postovulatory luteal phase rather than follicular. Assuming a 2.2 μg/L positive bias with SST, our current laboratory follicular phase progesterone reference range of <1.6 μg/L could increase to <3.8 μg/L with SST. We recommend that all progesterone serum samples be collected in plain glass tubes until this SST interference problem is resolved. Our laboratory has notified Roche Diagnostics of this finding.

References

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A representative of Roche Diagnostics responds:

To the Editor:
The authors pointed out that falsely increased test results of the Elecsys® Progesterone assay may occur if gel separation tubes are used. This effect was not seen with other assays of the Elecsys reagent line. It may be directly related to the serum volume and the exposure time of the specimen to the tube. We have received similar feedback from others. Our own investigations could not fully elucidate the process that leads to the interference, but as the authors correctly concluded, it might be caused by one of the gel components.

To solve this interference, Roche Diagnostics started to develop a new assay at the beginning of 1998, named Elecsys Progesterone II, which entered the market in April 1999. The Elecsys Progesterone II will have completely replaced the Elecsys Progesterone by the end of June 1999. The Elecsys Progesterone II exhibits a new test design with respect to anti-progesterone antibodies and reagent composition and eliminates the interference caused by gel separation tubes. In conclusion, the authors’ investigations describe a not clearly understood phenomenon obtained for one Elecsys steroid assay that is no longer commercially available.

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More on Calcium Ion Activity Buffers for Coagulation Testing

To the Editor:
The partial thromboplastin time (PTT) test has been problematic over the years, in part because of the multiple reagent and instrument systems used for this measurement. However, preanalytic variables are also operative. Standardization of the prothrombin time (PT) with the International Normalized Ratio has been difficult, but the more complex PTT has not yet been standardized even to this extent. Part of the problem is the greater number of variables that affect the PTT compared with the PT (1).

An article by Rånby et al. (2) in the most recent issue of Clinical Chemistry provided a detailed study of calcium ion buffers used for the collection of blood specimens for the PTT. Because the PTT is the coagulation test of choice to screen for coagulation abnormalities and to monitor heparin therapy, any measures to improve the test would be most welcome.

Some years ago I, and others in my laboratory, became interested in the so-called preinstrumental variables of coagulation testing, which included the effects of the higher than necessary concentrations of citrate anticoagulants in specimens with increased hematocrits (3). We proposed using a lower concentration of citrate, i.e., 109 mmol/L (32 g/L) sodium citrate anticoagulant rather than the traditional 129 mmol/L (38 g/L) (4), for blood collection; this lower concentration currently is recommended. Subsequent work in our laboratory included investigation of the concentration of citrate anticoagulant (which Rånby et al. refer to as buffer) and its effect on the PTT, especially in connection with heparin therapy. We showed that the concentration of citrate ions does influence PTT results, especially at higher heparin concentrations (5). Therefore, it was with more than usual interest that I read the studies of Rånby et al. (2), who propose the substitution of isocitrate for citrate when collecting specimens for coagulation studies. A more “chemically correct” anticoagulant might well improve the diagnostic usefulness of the PTT, but several questions come to mind that would have to be studied before serious consideration is given to modifying the blood collection anticoagulants/buffers for coagulation testing.

Most laboratories use the same collection methods for both the PT and PTT. Because both tests are done on the same plasma sample, the effect of isocitrate anticoagulant on the PT would have to be studied. Although there is no reason to believe that isocitrate would adversely affect PT testing, careful comparative studies would have to be carried out.

More importantly, Rånby et al. opine that the revised procedure would be more sensitive to mild coagulation factor deficiencies. According to their statistical studies of comparative reference intervals, there were more mildly abnormal PTTs with the proposed anticoagulant/buffer. However, the effect of isocitrate on specific coagulation factor assays has not yet been studied.

In our studies, we have reported the clinical correlation studies needed to confirm that these tests have correctly identified individuals with mild coagulopathies. Of course, an increased sensi-