samples collected by Salivette® (common for cortisol) are not significantly different from values in passive drool samples, indicating that the Salivette does not influence salivary transferrin values.

We speculate that endogenous peroxidase in saliva has the potential to cause false-positive (high) readings with methods that base their detection on this chemical reaction. A large component of the variance in the values determined with the Hemastix method with saliva may be measurement error.

We also found that mild to moderate injury to the oral mucosa rapidly (within minutes) increased visual inspection ratings of saliva discoloration, Hemastix values, and transferrin concentrations (7). Within 15 min of micro injury, sample discoloration ratings returned to baseline values. By contrast, salivary transferrin and Hemastix scores remained increased over baseline for 30 min. These findings underscore that blood components can be present in saliva even in the absence of visual evidence of blood contamination. The amount of blood contamination in response to this mild to moderate injury did not change either DHEA or cortisol, but increased salivary testosterone in samples not visibly contaminated.

Measured testosterone was increased when transferrin was ≥5 mg/L, and measured DHEA and cortisol were increased at transferrin >10 mg/L. Our findings suggest that an additional amount of care may need to be taken to ensure that salivary immunoassay results are accurate. The presence of blood components in saliva can be invisible to the eye and has potential to distort the true values of salivary analytes. There are distinct problems and pitfalls associated with measurement strategies used in the literature to screen and quantify blood contamination in saliva that limit their usefulness. Screening procedures may need to be used in the laboratory to rule out contamination of saliva with blood. We anticipate that awareness of these issues may help investigators and testing laboratories improve or expand the accuracy of salivary measurement.

We appreciate the technical, conceptual, and analytical assistance provided by Mary J. Curran, Laurel O’Brien, Ruth Merritt, Amber Baptiste, Elizabeth A. Shirtcliff, and Genevieve Shulick. This study was supported in part by the Pennsylvania State University Behavioral Endocrinology Laboratory and Salimetrics LLC. We acknowledge partial ownership in Salimetrics LLC.

References

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Comparison of Five Different Citrated Tubes and Their in Vitro Effects on Platelet Activation, Jan Philippe,* Erik De Logi, and Gaston Baele (Department of Clinical Chemistry, Microbiology and Immunology, University Hospital Ghent, De Pintelaan 185, B-9000 Ghent, Belgium; * author for correspondence: fax 32-9-2404985, e-mail jan.philippe@ugent.be)

After blood is collected in a tube, platelet activation occurs rapidly, hampering studies of other causes of platelet activation (1–3). Ideally, collection tubes for such studies have minimal activation potential. In vitro platelet activation has consequences for the evaluation of heparin activity as well (4) because activated platelets might release platelet factor 4 (PF4), inactivating heparin and leading to artificial shortening of activated partial thromboplastin time (APTT) results.

To evaluate platelet activation, markers appearing on the cell surface can be studied, as can the release of products from platelet α-granules. The purpose of this

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**Table 1. Mean (SE) increases in concentrations of cortisol, DHEA, testosterone, and transferrin in saliva samples with added blood.**

<table>
<thead>
<tr>
<th>Whole blood present, %</th>
<th>Mean (SE), nmol/L</th>
<th>Change, %</th>
<th>Mean (SE), nmol/L</th>
<th>Change, %</th>
<th>Mean (SE), nmol/L</th>
<th>Change, %</th>
<th>Mean (SE), mg/L</th>
<th>Change, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>3.23 (0.83)</td>
<td>37</td>
<td>0.60 (0.11)</td>
<td>180</td>
<td>0.22 (0.09)</td>
<td>259</td>
<td>36.6 (2.7)</td>
<td>1835</td>
</tr>
<tr>
<td>5</td>
<td>1.16 (0.28)</td>
<td>13</td>
<td>0.28 (0.05)</td>
<td>82</td>
<td>0.13 (0.06)</td>
<td>155</td>
<td>20.2 (1.5)</td>
<td>1010</td>
</tr>
<tr>
<td>2.5</td>
<td>0.28 (0.14)</td>
<td>3</td>
<td>0.08 (0.03)</td>
<td>24</td>
<td>0.06 (0.03)</td>
<td>72</td>
<td>11.2 (1.0)</td>
<td>560</td>
</tr>
<tr>
<td>1.25</td>
<td>0.01 (0.07)*</td>
<td>3</td>
<td>0.03 (0.01)</td>
<td>33</td>
<td>0.01 (0.005)</td>
<td>10</td>
<td>3.4 (0.4)</td>
<td>170</td>
</tr>
<tr>
<td>0.63</td>
<td>0.00</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
<td>0.63</td>
<td>0</td>
</tr>
</tbody>
</table>

* Not a significant change.
Study was to evaluate the effect of blood sampling tubes on platelet activation. We compared five tubes:

- VT-050SBCS07 [Terumo Venoject® glass, containing 0.5 mL of buffered sodium citrate solution (32 g/L; final concentration, 0.109 mol/L), hereafter called tube 1;VF-055SBCS07 [Terumo Venosafe® plastic, containing 0.4 mL of buffered sodium citrate solution (32 g/L; final concentration, 0.109 mol/L), hereafter called tube 2; VF-053SBCS07 [Terumo Venosafe plastic, containing 0.3 mL of buffered sodium citrate solution (32 g/L; final concentration, 0.109 mol/L)], hereafter called tube 3; BD363079 [BD Vacutainer® Plus, containing 0.3 mL of sodium citrate solution (final concentration, 0.129 mol/L) in a plastic coagulation tube]; hereafter called tube 4; and
- GR454332 [Greiner Vacuette® Coagulation plastic tube, containing 0.35 mL of sodium citrate solution (32 g/L; final concentration, 0.109 mol/L)]; hereafter called tube 5.

Because there is a 9:1 blood: citrate volume ratio in the tubes, the final citrate concentration after tubes are filled with blood is 0.0109 mol/L in all tubes except for tube 4, which has a final concentration of 0.0129 mol/L.

We measured CD62, or P-selectin (GMP-140; PAD-GEM), which is a platelet α-granule protein expressed on the surface of platelets after activation and is used to characterize platelet activation. In this study we measured the percentage of platelets positive for CD62 by measuring an isotypic control in parallel. Phycocerythrin-conjugated monoclonal antibody CD62 (clone AC1.2) was purchased from BD Biosystems. Platelets were gated by using CD61 antibody labeled with FITC (clone RUU-PL7F12; BD Biosystems). Analysis was performed on a FACSort flow cytometer (BD Biosystems).

We measured PF4 and β-thromboglobulin (β-TG), soluble released markers of platelet activation, by enzyme immunoassays from Asserachrom and Diagnostica Stago, respectively.

We collected 16 tubes of blood from each of 30 healthy volunteers who had taken no medication for at least 2 weeks. The first (plain) tube was discarded. Triplicates of each of the five tubes were then drawn. The order of tubes was randomized throughout the study. After sampling, tubes were turned top down and then upright five times slowly. One tube per triplicate was used to measure CD62 at 10, 60, and 120 min after sampling. A second tube was cooled immediately at 4 °C after sampling and centrifuged at 2500g for 30 min. Platelet-poor plasma was then transferred to a secondary tube and frozen at −80 °C until further analysis. A third tube was cooled at 4 °C and after 120 min was centrifuged at 2500g; the platelet-poor plasma was then stored at −80 °C until analysis. PF4 and β-TG were measured in all stored plasma samples.

The results over time for the five tubes were analyzed by use of a repeated-measures model with SAS software (SAS Institute Inc.). Because one of each test tube was drawn from each volunteer, the “tube” effect was considered as a repeated effect, and this was taken into account in the analysis. In addition, “time” was considered as a repeated, continuous effect in these models. Contrasts were made to test differences among the tubes. The results are plotted as the mean (SE) in Fig. 1.

Initial β-TG (Fig. 1, top) differed significantly (P = 0.0004) among tubes, and there was a significant time effect (P < 0.0001) for all tubes. The increase over time was different for the five tubes (P = 0.01). Tube 1 had significantly lower initial values than the four other tubes (P < 0.01 for all comparisons). Tubes 2, 3, 4, and 5 had similar initial values. The increase over time of β-TG in tube 1 was not significantly lower than the increase over time in tube 2 but was significantly lower than the increase over time in tubes 3, 4, and 5. The increase over time of β-TG in tube 2 was significantly lower than the increase over time in tube 5. The highest rate of increase (in tube 5) was ~0.92 (0.11) IU·mL⁻¹·min⁻¹.

For PF4 (Fig. 1, middle), there was a significant main tube effect (P < 0.02), i.e., there were differences in initial values. Tube 1 had the lowest initial values, whereas tubes 2, 3, 4, and 5 started off with higher concentrations (Fig. 1). The calculated estimates for some predefined tube contrasts (comparisons) showed that tube 1 had significantly higher values than the other tubes.

![Fig. 1. Mean (SE; error bars) values for the five tubes as described in the text for β-TG (top) and PF4 (middle) 5 and 120 min after sampling, and for CD62 (bottom) 10, 60, and 120 min after sampling (n = 30).](image_url)
lower starting values than tubes 3 and 4, but had values similar to those in tubes 2 and 5.

PF4 changed over time in all of the tubes \((P < 0.0001)\). The interaction effect was also significant, indicating a difference among the five tubes for changes in PF4 over time \((P < 0.0001)\). The increase with time was significantly lower in tube 1 than in tube 2 \([0.18 (0.09) \text{ lower IU} \cdot \text{mL}^{-1} \cdot \text{min}^{-1}]\), tube 3 \([0.21 (0.09) \text{ lower IU} \cdot \text{mL}^{-1} \cdot \text{min}^{-1}]\), tube 4 \([0.32 (0.09) \text{ lower IU} \cdot \text{mL}^{-1} \cdot \text{min}^{-1}]\), and tube 5 \([0.45 (0.09) \text{ lower IU} \cdot \text{mL}^{-1} \cdot \text{min}^{-1}]\). Furthermore, tubes 2 and 3 showed a similar time-wise increase, significantly lower than tube 5 \([-0.27 (0.09) \text{ IU} \cdot \text{mL}^{-1} \cdot \text{min}^{-1}; P = 0.003]\).

For CD62 expression (Fig. 1, bottom), all of the tubes had similar initial values. The repeated-measures analysis did not show a main tube effect \((P = 0.99)\), indicating that all tubes started at similar values. All tubes showed a similar increase in CD62 expression. After 60 min, there was an additional, but smaller, increase in CD62 in all tubes up to 120 min, except for tubes 1 and 5, which had a slight decrease from 60 min to 120 min. There was a significant linear time effect \((P < 0.0001)\) and quadratic time effect \((P = 0.001)\), i.e., the values changed over time, and there was a significant curvature over time (the increase was not a straight line). These time effects were similar for the five tubes (both interactions not significant, \(P = 0.83 \text{ and } 0.67\), respectively).

For PF4 and \(\beta\)-TG, therefore, there were significant initial \((t5)\) differences. For PF4, the initial values for tube 1 were significantly lower than those for tubes 3 and 4 and were not significantly lower than the values for tubes 2 and 5. For \(\beta\)-TG the values for tube 1 were significantly lower than those of all other tubes. There were significant differences among the tubes in the increase over time for both analytes. Tube 5 had the highest increase. Tubes 4 and 5 had similar rates of increase. Tube 1 had by far the lowest increase. The pattern for PF4 was very similar to that for \(\beta\)-TG and similar for the five tubes.

For CD62, there were no significant initial differences in CD62 values among the tubes, there was a significant linear time effect and a significant quadratic time effect, there were no significant differences among the tubes for the increase over time, and all tubes showed an increase at first, followed by a negative quadratic curvature. PF4 and \(\beta\)-TG are continuous variables, whereas CD62 is a categorical variable. The latter cannot distinguish between CD62 weakly positive and CD62 strongly positive platelets. This may explain the lack of correlation between the two kinds of variables.

We conclude that for studies of platelet activation, the glass tube performed best. The plastic tubes did not differ greatly, except for a slightly better performance of the VF-054SBCS07 Terumo Venosafe tube. With respect to the evaluation of heparin activity by APTT, one might select the two above-mentioned tubes on the basis of the least increase in PF4 over time. It is clear that to avoid a significant inhibiting effect of PF4 on heparin activity, analysis should be performed within a short time after sampling.

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**References**


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**Electrochemical DNA Array for Simultaneous Genotyping of Single-Nucleotide Polymorphisms Associated with the Therapeutic Effect of Interferon**, Masayoshi Takahashi,1,2 Jun Okada,1 Keiko Ito,1 Michie Hashimoto,1 Koji Hashimoto,1 Yuri Yoshida,2 Yasuhiro Furuichi,2 Yasuhiko Ohta,2 Shunji Mishiro,3 and Nobuhiro Gemma1 (1 Toshiba Research & Development Center, 1, Komukai Toshiba-cho, Saiwai-ku, Kawasaki, Kanagawa 212-8582, Japan; 2 GeneCare Research Institute Co., Ltd., Kamakura-shi, Kanagawa, Japan; 3 Toshiba General Hospital, Shinagawa-ku, Tokyo, Japan; author for correspondence: fax 81-44-549-2426, e-mail masayoshi8.takahashi@toshiba.co.jp)

Approximately 170 million people worldwide are affected by the hepatitis C virus (HCV). Interferon has been developed to treat HCV hepatitis, but its effectiveness depends on factors including the type and copy number of the infecting viruses and individual patient characteristics. Some single-nucleotide polymorphisms (SNPs) in the host are correlated with responsiveness \((1–4)\). Two SNPs are at nucleotide positions \(-88 (G \text{ or } T) \text{ and } -123 (C \text{ or } A)\) within an interferon-stimulated response element-like sequence in the promoter region of the \(MxA\) gene \((1, 2)\). The MxA protein is interferon-inducible and is known to inhibit the replication of a wide variety of single-stranded RNA viruses \((5)\). Two other of these SNPs are at nucleotide position \(-221 (C \text{ or } G; X/Y)\) within the promoter region of the manno-binding lectin \((MBL)\) gene and at codon 54 \((A \text{ or } G; A/B)\) within exon 1 of this gene \((3, 4)\). The functions of MBL include the elimination of pathogens \((6)\). The identification of these genetic variations in patients can help predict the efficacy of interferon in the treatment of HCV.

DNA microarrays or DNA chip-based technologies can be used for the simultaneous genotyping of polymorphisms. The technologies used in recently reported DNA hybridization devices or indicators include gold nanoparticles \((7, 8)\), enzyme-amplified electronic transduction \((9)\),