reflect the slightly higher variability in the agreement between serum and blood spot results for samples <1.0 mg/L. There was no evidence of systematic differences in the serum:blood-spot CRP ratio across the assay range.

Hematocrit correction does not improve agreement between plasma and blood-spot results for gonadotropins and is not necessary for samples with normal hematocrits (12). To confirm this for CRP, we added washed erythrocytes (in concentrations of 30%, 40%, and 50%) to three plasma samples and spotted them on filter paper. There was no consistent association between hematocrit and blood-spot CRP concentration.

Many epidemiologic analyses of the association between serum CRP and cardiovascular disease risk are categorical, with the distribution of CRP concentrations divided into tertiles or quartiles (7, 9). Recently, cutpoints of low risk (<1.0 mg/L), average risk (1.0–3.0 mg/L), and high risk (≥3.0 mg/L) have been proposed that approximate the tertile distribution of serum/plasma CRP in a range of populations (10). We compared category assignments according to the blood-spot and serum methods in our 94 matched samples, with the distributions of blood-spot and serum CRP concentrations divided separately into tertiles. Eighty-seven of 94 individuals (93%) were assigned to the same category by both methods.

We evaluated the stability of CRP in dried blood spots by exposing nine samples sealed in plastic bags with two dessicant packs (cat. no. 61161-319; VWR) to the following temperature conditions for up to 14 days: 37°C, 4°C, and −20°C. An additional variable-temperature condition was evaluated (12 h at 32°C and 12 h at 22°C) to simulate ambient temperature exposures in tropical environments. Samples were considered to remain stable as long as CRP concentrations remained within 2 SD of baseline values measured in samples stored at −30°C immediately after collection. Six determinations were used to calculate the baseline mean (SD) for each sample: 1.11 (0.10), 1.58 (0.093), 1.60 (0.15), 2.46 (0.25), 2.65 (0.17), 4.28 (0.17), 4.57 (0.32), 6.40 (0.55), and 6.64 (0.44) mg/L. CRP concentrations remained stable in dried blood spots for 3 days at 37°C, 3 days at 32/22°C, and for at least 14 days at room temperature and 4°C. The stability of CRP to repeated cycles of freezing and thawing was also evaluated, with no evidence of deterioration after five freeze-thaw cycles (1 h at room temperature, repeated over 5 different days).

Previous applications of whole-blood-spot methods have demonstrated performance characteristics similar to those for methods that rely on venipuncture (17). The ease of finger stick blood collection alleviates constraints associated with sampling in clinical settings, increases the frequency with which samples can be taken, and expands the methodologic options for population-level health research. In particular, our high-sensitivity CRP method could potentially be a useful tool for community-based, epidemiologic investigations of inflammation and cardiovascular risk.

This study was approved by the Institutional Review Board of the Evanston Northwestern Healthcare Research Institute. Financial support provided by the National Science Foundation (BCS-0134225).

References

DOI: 10.1373/clinchem.2003.029488

Transferrin Enzyme Immunoassay for Quantitative Monitoring of Blood Contamination in Saliva, Eve E. Schwartz* and Douglas A. Granger† (Salimetrics LLC, State College, PA; ‡Behavioral Endocrinology Laboratory, Department of Biobehavioral Health, Pennsylvania State University, University Park, PA; *address correspondence to this author at: Salimetrics LLC, 101 Innovation Blvd., Suite 302, State College, PA 16803; fax 814-234-1608, e-mail lbs@salimetrics.com)

When blood components are present in the oral mucosa, quantitative estimates of salivary hormone concentrations may be compromised (1). Blood and its components can
leak into the oral mucosa as a result of micro injuries such as burns or abrasions. The probability of blood leakage is increased with poor oral health (i.e., periodontal disease), certain infectious diseases (e.g., HIV) (2), and behavior known to influence oral health negatively (e.g., smoking) (3). Few studies, however, have investigated methods to determine blood contamination in saliva.

The Hemastix® Reagent Strip for Urinalysis has been used to test for blood in saliva (4, 5). This method detects the pseudoperoxidase activity of hemoglobin; thus, endogenous salivary peroxidases produce false-positive results for hemoglobin.

To provide a quantitative measure of blood contamination in saliva, we developed an enzyme immunoassay for transferrin, which is present in very small amounts (<5 mg/L) in saliva and in high concentration in whole blood (>1000 mg/L). We expect that serum and salivary concentrations of transferrin are not correlated and that transferrin, with a formula weight of 76 000 and radius of 5 nm, would reach saliva in high concentrations only when the barrier between blood and saliva is compromised.

Saliva (by passive drool) and serum samples were collected without stimulation simultaneously from 40 young adults. The Pennsylvania State University Institutional Review Board approved all procedures, and informed consent was obtained. All samples were stored at −40 °C. All samples were thawed, vortex-mixed, and centrifuged at 1500g for 15 min before assay for serum transferrin, salivary transferrin, or testing by Hemastix Reagent Strip.

Blood-contaminated saliva from three men and three women was constructed by dividing saliva samples into six 0.45-ml aliquots. One saliva sample from each individual was used as the control (no blood added). On the day of collection, 50 μL of blood was added to one aliquot of that individual’s saliva, and this contaminated saliva was serially diluted twofold into the remaining five aliquots. These samples represented 0.63–10% whole blood in saliva.

Salivary transferrin was measured by enzyme immunoassay based on a competitive reaction between transferrin and serum transferrin linked to horseradish peroxidase (Salimetrics LLC).

Hemastix Reagent Strips use 3,3′,5,5′-tetramethylbenzidine and diisopropylbenzene dihydroperoxide. One minute after sample addition, the strip color is compared with the color chart, and the closest match is recorded. We recorded readings as “negative” through 4.

We measured salivary dehydroepiandrosterone (DHEA) and cortisol by immunoassays (Salimetrics LLC). Intra- and interassay imprecisions (CV) were 6.8% and 6.5% for DHEA and 5.1% and 8.2% for cortisol. The detection limits, calculated by interpolating the mean minus 2 SD of the mean of 20 results for the zero calibrator, were 0.035 nmol/L for DHEA and 0.083 nmol/L for cortisol. Salivary testosterone was measured by a modification (6) of the Diagnostic Systems Laboratories double-antibody RIA. The intra- and interassay CV were 5.7% and 6.3%, with a detection limit of 0.003 nmol/L.

Serum transferrin was measured by radial immunodiffusion assay (Kent Laboratories). The antibody gives a single line of identity to transferrin against whole human serum by immunoelectrophoresis.

Radioactivity was measured on a Wallac model 1272 gamma counter with data reduction by Term 2 software (Perkin-Elmer Life Sciences). Absorbances were read on a Sunrise plate reader (Tecan) with data reduction by Assayzap software (Biosoft).

The mean (SE) slope (n = 20) of the calibration curve was 0.92 (0.01) [range, 0.878–0.988; r = 0.9994–0.9999]. The mean (SE) estimated dose at 20% bound (ED20) was 5.8 (0.2) mg/L, the ED50 was 1.2 (0.05) mg/L, and the ED80 was 0 (0.0) mg/L. The detection limit was 0.8 mg/L. The range of the calibration curve was 0.8–66.0 mg/L transferrin. Mean (SE) recovery of transferrin (3.3–50 mg/L) added to saliva was 100 (4.7)% (range, 88.9–110.4%). Intraassay imprecisions (CV; n = 20) were 5.4%, 7.4%, and 7.4%, respectively, at 35, 9, and 2.5 mg/L. Interassay CV computed from the mean of average duplicates for 20 separate runs were 5.4%, 6.0%, and 13%, respectively, at similar concentrations. To evaluate assay linearity, we added transferrin (50 mg/L) to saliva and serially diluted to 16-fold. Linear regression analysis revealed a slope of 0.995 and mean (SE) intercept of −0.007 (0.047) mg/L (S_{y|x} = 0.007 mg/L; r^2 = 0.9995).

Antibody specificity was evaluated by testing testosterone, cortisol, DHEA, cortisone, corticosterone, 11-deoxy cortisol, 21-deoxycortisol, prednisolone, prednisone, dexamethasone, triamcinolone, 17-α-hydroxyprogesterone, estradiol, aldosterone, progesterone, and estradiol at 50 μg/L or higher. Cross-reactivity was <1% for all analytes. Serum and saliva concentrations of transferrin were not significantly correlated (r = 0.03; P = 0.851; n = 39).

Transferrin was <4.0 mg/L in the saliva from six volunteers. The mean (SE) initial values for cortisol, DHEA, and testosterone were 8.66 (1.26) nmol/L, 0.34 (0.11) nmol/L, and 0.09 (0.02) nmol/L, respectively. When blood was added to saliva at concentrations of 0.63–10%, transferrin concentrations increased proportionately. Measured cortisol, DHEA, and testosterone values also increased in saliva when blood was present. Differences were observed in acceptable amounts of blood contamination depending on which analyte was measured: >10 mg/L transferrin caused increases in cortisol and DHEA, whereas testosterone was increased at transferrin >5 mg/L (Table 1).

Transferrin concentrations and Hemastix values in 20 saliva samples were not significantly correlated (r = 0.32; P = 0.46). Hemastix indicated blood contamination in 19 of 20 samples, whereas the salivary transferrin was <5 mg/L. In the four samples with the highest Hemastix values (4), suggesting a large amount of blood present, transferrin was <2 mg/L.

These results suggest that salivary transferrin can serve as a surrogate marker for quantification of blood contamination in saliva. Mean salivary transferrin values in
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 Salimeters LLC. We acknowledge partial ownership in Salimeters LLC.

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<th>Mean (SE), nmol/L</th>
<th>Change, %</th>
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a Not a significant change.

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

References

DOI: 10.1373/clinchem.2003.028266

Comparison of Five Different Citrated Tubes and Their in Vitro Effects on Platelet Activation. Jan Philippe,* Erik De Logi, and Gaston Baelde (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 artifactual 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...