for glucose, calcium, chloride, and uric acid—as demonstrated by the kurtosis and skewness coefficients and by the Kolmogorov–Smirnov test.

The fetal reference intervals for sodium, potassium, urea nitrogen, creatinine, lactate dehydrogenase, uric acid, and magnesium are very close to those of infants. For glucose, aspartate aminotransferase, alanine aminotransferase, cholesterol, and triglycerides, the fetal reference intervals are shifted to much below those of adults. The fetal reference interval for total bilirubin is lower than that of infants but higher than that of adults.

The lower limit of the fetal reference interval for calcium was below that of adults, but the upper limits of the fetal reference interval for chloride, inorganic phosphorus, and direct bilirubin were higher. The interval for enzymatic activity of γ-glutamyltransferase was higher than that in both infants and adults.

Recently, Perkins et al. [8] reported reference intervals for 21 clinical chemistry analytes determined in plasma obtained from arterial and venous umbilical cord blood from healthy term infants after delivery but before placental expulsion. Except for K, Ca, P, Cl, uric acid, and γ-glutamyltransferase enzymatic activity, our findings are similar to those reported for healthy term infants. We are not able to fully explain these differences; presumably they are related to the different size and composition of the fetal reference populations, given that our adult reference intervals are closely similar to those of Perkins et al.

In conclusion, the analysis of our data obtained for 18 analytes suggests that the fetal reference intervals are similar to those of infants only for urea nitrogen, Na, K, creatinine, lactate dehydrogenase, uric acid, and Mg. For those analytes that change as a function of gestational age, knowledge of the fetal reference intervals according to stage of pregnancy is important, particularly when therapy is necessary (e.g., albumin infusions in nonimmune hydrops).

Preanalytical Determinants of Total and Free Prostate-Specific Antigen and Their Ratio: Blood Collection and Storage Conditions, Klaus Jung,* Philipp von Klinggräff, Brigitte Brix, Pranav Sinha, Dietmar Schnorr, and Stefan A. Loening (1Dept. of Urol. and 2Inst. of Pathological and Clin. Biochem., Univ. Hosp. Charité, Humboldt Univ. Berlin, Schumannstr. 20/21, D-10098 Berlin, Germany; *author for correspondence: fax +49 30 2802 1402, e-mail jung@rz.charite.hu-berlin.de)

Prostate-specific antigen (PSA) is present in serum in several forms [11]. About 70–90% of total PSA (t-PSA) is
complexed with serum protease inhibitors, especially with α1-antichymotrypsin. About 10–30% are not bound to serum proteins (at least not with high affinity), and that fraction is called free PSA (f-PSA). Patients with prostate cancer exhibit a lower ratio of f-PSA to t-PSA (f-PSA%) than patients with benign prostatic hyperplasia [1]. Subsequent studies have shown the clinical usefulness of f-PSA% in distinguishing between these two groups of patients [2]. Whereas preanalytical, analytical, and biological factors of t-PSA changes have already been compiled [3–5], details on variation of f-PSA% are still lacking. The influence of preanalytical factors like blood collection, storage conditions, and freeze-thaw cycles on that ratio is of special interest because PSA as a nonurgent analyte is often quantified in batches after various storage periods [6]. The purpose of the present study is to gain insight into these influencing factors on PSA fractions as measured by a widely used instrument and to lay down practical recommendations.

AxSYM PSA and AxSYM Free PSA (Abbott Diagnostics), automated microparticle enzyme immunoassays, were used for measuring t-PSA and f-PSA, respectively [7]. Each run was checked by three control sera for t-PSA (4.06, 14.6, and 45 μg/L) and f-PSA (0.38, 0.98, and 6.84 μg/L). The between-run CVs (n = 32) were 3.7–4.5% for t-PSA and 2.9–3.8% for f-PSA. The within-run CVs (n = 12) were 2.1–3.5%.

The study included 18 patients with prostate cancer and 4 patients with benign prostatic hyperplasia. All procedures followed were approved by the Ethical Standard Committee of the hospital. The t-PSA was 3.47–77.3 μg/L (median 12.4 μg/L), f-PSA 0.5–13.5 μg/L (median 1.26 μg/L), and f-PSA% 1.1–30.5% (median 9.04%). All values were above the lower detection limits (means ± SD; 10 replicate intraassay determinations of the zero calibrators) for t-PSA and f-PSA of 0.096 μg/L and 0.005 μg/L, respectively.

Blood samples were collected in evacuated tubes (Sarstedt, Monovette 03.1528). After allowing the blood to clot for 1 h at room temperature, the samples were centrifuged (1600g, 15 min at 4 °C). Statistical calculations

![Fig. 1. Stability of fPSA, t-PSA, and ratio of fPSA/t-PSA in serum at different storage temperatures depending on storage time.](image)

All values were given as percentage arithmetic means ± SD of the initial values. Significant differences (at least *P < 0.05*) compared with the starting value calculated by one-way ANOVA test for repeated measures followed by the posttest of Dunnett are indicated by asterisks (*).
were performed with GraphPad Prism (GraphPad). One-way ANOVA test for repeated measures followed the posttests of Dunnett and of linear trend. $P < 0.05$ was considered statistically significant.

Figure 1 shows the behavior of the analytes and the ratio depending on different storage temperatures. Although the storage of serum at 37°C (Fig. 1A) for 1 h already reduced the initial t-PSA and f-PSA values significantly by about 5%, the mean decrease did not exceed 10%, even after 24 h of storage at 37°C. F-PSA and t-PSA values decreased similarly for all time intervals out to 24 h, with little effect of the f-PSA%. Under storage at 22°C and 4°C (Fig. 1B, C), f-PSA values showed a distinctly greater decrease than t-PSA values as storage intervals increased. The decrease of about 5–7% both for t-PSA and f-PSA became significant after 4 h at room temperature and after 24 h at 4°C with no effect of f-PSA%. Storage of serum at −80°C (Fig. 1D) did not alter f-PSA, t-PSA, and their ratio over 4 months.

To study the effect of sample preparation, venous blood samples simultaneously collected in plastic tubes for preparation of serum and in lithium heparin-coated plastic tubes for preparation of plasma (Sarstedt) were stored at room temperature for different time periods (1, 2, 4, 8, and 24 h). After centrifugation, the supernatants were stored at −80°C until analysis in a single run. Whereas t-PSA values were stable over 24 h, a significant mean decrease of about 5% and 8% was observed for f-PSA and consequently for the ratio after 8 h and 24 h, respectively. We also tested the stability in heparin plasma and found similar results, i.e., only f-PSA decreased significantly by about 7% when the blood was stored in heparin-coated plastic tubes over 30 h before centrifugation.

To study the effect of freeze–thaw cycles, sera frozen at −80°C were subjected to 1–5 freeze–thaw cycles at room temperature for 2 h, mixed, and then refrozen and analyzed together after the last freeze–thaw cycle. The stability of f-PSA and t-PSA was only slightly affected by the freeze–thaw process and confirmed data of other authors [8]. Five cycles reduced the initial value of f-PSA by only about 5%.

Our results show that specimen handling and storage conditions affect f-PSA and t-PSA, in different ways. The general recommendations of sample storage for t-PSA [6, 9, 10] do not apply to f-PSA. Storage of blood before serum separation and storage of serum at room temperature or 4°C showed that f-PSA was less stable than t-PSA. Two effects have to be considered for the decreased values. Either the immunoreactivity of the respective analyte is really affected or the changes result from alterations in the binding affinity/dissociation behavior of PSA to the above-mentioned protease inhibitors or other ligands. However, both mechanisms may change the apparent stability of serum f-PSA and t-PSA in a combined fashion. Moreover, since the relatively greater decrease in f-PSA than t-PSA values was especially observed at 4°C, a nonspecific effect, e.g., due to the complexation of f-PSA with serum substances, not necessarily protease inhibitors, may be also possible. In this respect, these results confirm similar data recently published elsewhere [11]. Our stability results of f-PSA and t-PSA at −80°C proved that the mentioned changes did not take place under these conditions for at least 4 months. However, as described above there were few individual samples with f-PSA values slightly reduced already after 4 months storage. Running stability studies will be necessary. Storage at −80°C should be preferred to storage at −20°C since serum samples showed decreased t-PSA concentrations at that temperature after 3 weeks [10].

Whatever the mechanisms of these changes under different conditions of storage and sample preparation may be, our results imply the following practical guidelines to limit the effect of preanalytical factors as interfering variables on the measurement of t-PSA, f-PSA, and f-PSA%:

- Serum should be separated from cellular elements within 4 h after venipuncture.
- Samples to be run on the same day are best kept at 4°C, as samples stored at room temperature showed a significant loss of immunoreactivity within 4 h.
- Samples that cannot be analyzed within 8 h after collection can be stored at −80°C for at least 4 months.
- Three freeze–thaw cycles do not alter the stability of f-PSA and t-PSA.

While this study was performed, two resembling reports on this topic have been published [11, 12]. The authors obtained results similar to ours, although different PSA assays were used (Hybritech; Delfia). Thus, our guidelines for sample preparation and storage appear to be valid for other widely used tests for f-PSA and t-PSA. However, the clinical chemist and the physician should be aware that new assays should be tested for effects of preanalytic factors on the respective tests.

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References

The use of plasma fibrinogen concentrations to predict clinical ischemic events in subjects with and without prior evidence of atherosclerosis [1–14] has led to recommendations to incorporate fibrinogen into the cardiovascular risk profile [15]. Such use of plasma fibrinogen requires careful attention to potential sources of variability, as only small (5%) absolute differences in fibrinogen distinguish subjects who develop cardiovascular disease from those subjects who do not [16].

Variability in fibrinogen results apart from intrindividual differences is difficult to quantify, because the few groups who have examined these issues used different assays for fibrinogen determination. Moreover, the functional assay for fibrinogen is highly influenced by technician expertise and manipulation [17]. Previously, we examined within-run analytical CV, within-person, within-day biological variation, and a 6-week within-person biological variation with the Clauss method for fibrinogen determination [16].

We describe here selected effects of sample collection and storage on fibrinogen results and we propose guidelines for sample collection and storage.

Blood was collected in evacuated tubes (5-mL capacity) containing 0.5 mL of 0.129 mol/L (3.8%) buffered citrate solution (Becton Dickinson). Fibrinogen concentrations were determined by thrombin clottable time with the Clauss method (Dade Data-Fi, Baxter Diagnostics) [18] with bovine thrombin (100 kU/L) and 2.8 × 10⁻³ mol/L sodium barbital in 0.125 mol/L sodium chloride. Duplicate plasma samples were diluted 1:10 (by vol) with an imidazole buffer (Organon Teknika) and measured at 20- and 40-s intervals at 37 °C in a fibrometer (Model BBL, Becton Dickinson).

All samples were acquired from healthy, nonsmoking volunteers who were seated ≥5 min before phlebotomy [19]. Volunteers were excluded if they had current illnesses, regular and recent use of medications during the preceding 3 months, or an acute-phase illness in the preceding 2 months [20].

The impact of tourniquet pressure and duration was evaluated in a cross-over study design on 38 subjects. The tourniquet pressure was standardized with a sphygmomanometer and a C-clamp that was applied to the tubing to maintain the specified pressure. Mean arterial pressure (MAP) was calculated from three blood pressure recordings obtained at 1-min intervals from seated volunteers. Low pressure was defined as 40 mmHg tourniquet pressure. High pressure was calculated as the MAP + 10 mmHg (MAP + 10) for each subject. Fibrinogen samples were procured at three intervals of equal duration: 0–1 min, 2–3 min, and 4–5 min. For each interval, three replicate samples were acquired at each interval, and the mean value is reported.

On the basis of the findings of the tourniquet pressure and duration study, 14 consecutive blood samples were acquired from each of 38 subjects at low pressure within 1 min. All but the first acquired sample (which was discarded) were centrifuged to obtain plasma specimens. The first four plasma samples were delivered to the Coagulation Laboratory for analysis within 10 min. The remaining nine plasma samples were refrigerated at 4 °C. Three sets of plasma samples were delivered to the Coagulation Laboratory at 6 h, 24 h, and 48 h.

All statistical analyses were performed with SAS statistical package (ver. 6.10) [21], except for the Friedman test performed with SPSS for Windows [22]. Results are expressed as mean ± SD. The first-stick phenomenon was evaluated with a two-tailed paired t-test. The Friedman test was used to examine the influence of the duration of the tourniquet application on fibrinogen concentrations at low and high tourniquet pressures, and the influence of storage time on fibrinogen concentrations. The fibrinogen concentrations at each tourniquet pressure were compared at each time interval by using the paired-samples sign test. A 0.05 significance level was used for statistical tests. A critical difference or relative change value in fibrinogen measurement was defined as 5% on the basis of prospective population studies [16]. The percentage of subjects with a difference in fibrinogen values ≥5% was determined at low tourniquet pressure that was applied for 0–1 min, 2–3 min, and 4–5 min.

The first-stick phenomenon was evaluated in 115 subjects by comparing the fibrinogen concentration from the first-acquired sample (2.58 ± 0.47 g/L) with the average fibrinogen concentration of the next four samples (2.57 ± 0.47 g/L). No statistically significant difference was observed (P = 0.73).

The application of low tourniquet pressure was associated with a progressive increase in the concentration of plasma fibrinogen (P < 0.00005) across the three sample acquisition time periods (Table 1). The fibrinogen concentrations were significantly higher for samples acquired during intervals at 2–3 min (2.68 ± 0.70 g/L, P < 0.00005) and 4–5 min (2.70 ± 0.71 g/L, P = 0.0005) compared with those acquired during the first minute (2.62 ± 0.63 g/L).