shown that PSA is a widespread biochemical marker produced by many tumors, by normal tissues, and during pregnancy [2–5] and have pointed out the lack of specificity for prostate cells [6]. In light of evidence indicating the immunoreactivity of PSA in salivary gland neoplastic tissues [7], we undertook to study the PSA distribution in plasma and expression in saliva of a control group of healthy female volunteers. The PSA determinations were carried out with a commercially available kit from CIS Bio International (Gif-sur-Yvette, France), based on a solid-phase two-site IRMA. The CIS PSA-RIA CT assay utilizes two monoclonal antibodies, prepared against sterically remote sites of human PSA; the first is coated onto a test tube, the other is radionuclide labeled with $^{125}$I, and both sandwich the PSA molecules of the samples. The radioactivity bound to the test tube is linear in the range 0–120 μg/L, with a lower detection limit of 0.02 μg/L [8].

Blood and saliva were sampled from 20 apparently healthy women, ages 19–54 years (mean 34.3 years). Each participant was asked to rinse her mouth with water 5 min before collecting each sample. The saliva samples were centrifuged at 3500g for 15 min at 4 °C and the supernates were immediately frozen at −20 °C and thawed only once before assay. Blood samples were also drawn from these women at the time of the saliva collection and, after clotting, were centrifuged at 1500g for 15 min at 4 °C and then stored at −20 °C until assayed. Another 20 healthy women, ages 22–43 (mean 33.5 years), had been taking for at least the preceding 9 months a widely prescribed oral contraceptive containing 0.5 mg of gestoden (a progestin) and 0.035 mg of ethinylestradiol per tablet. The present work was carried out in accordance with the ethical standards of the Helsinki Declaration of 1975, as revised in 1983.

All samples examined contained detectable amounts of PSA, with a median of 0.039 μg/L. No statistically significant difference in plasma concentration was found between the control women and the group that had been taking the hormonal contraceptive. We also found no statistically significant correlation between PSA content and the women’s age, even after logarithmic transformation of data. Of the 40 women, 34 (85%) showed a plasma PSA concentration ≤0.06 μg/L—results in close agreement with those reported previously [5]. The PSA concentrations in saliva correlated significantly with the plasma concentrations in the healthy subjects ($r = 0.684, Z = 3.447, P = 0.006$), but in women taking progestin-containing oral contraceptives the saliva PSA content was significantly greater than the concentrations in plasma (see Table 1).

To our knowledge (after a careful review of the literature) this is the first report concerning the concentrations of PSA in saliva. A previous immunohistochemical study revealed a positive stain for this antigen in the brush border of ductal epithelial cells of normal salivary gland, as well as in several types of salivary gland tumors (i.e., adenoma/carcinoma of human salivary gland) [7].

The detectable amounts of PSA in saliva from healthy women give further evidence of the distinctiveness of PSA as a widespread biochemical marker. The increase of oral-contraceptive-induced PSA expression is probably related to modulation of this serine protease by several hormones [9, 10]. The presence of progesterone receptors in human salivary gland [11] may represent one possible mechanism of increased stimulation of PSA expression in saliva of women receiving oral hormonal contraceptive.

The biological effects and the mechanism causing the increase, however, remain difficult to explain. The possible biological role of this serine protease in nonprostatic tissue as a potential sensitive molecular marker of hormone responsiveness of the glandular cells, as yet only hypothesized [10], must be further investigated.

Table 1. Plasma and saliva PSA values in women.

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SE (and range), μg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>Saliva</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>0.047 ± 0.008</td>
</tr>
<tr>
<td>(0.02–0.16)</td>
<td>(0.02–0.15)</td>
</tr>
<tr>
<td>Contraceptive takers</td>
<td>0.045 ± 0.006</td>
</tr>
<tr>
<td>(0.02–0.12)</td>
<td>(0.045–0.34)</td>
</tr>
</tbody>
</table>

* Significantly different by paired t test from saliva concentrations of controls ($P = 0.0038$) and from their own paired plasma concentrations ($P = 0.0101$).

n = 20 in each group.

References


Four Automated Methods for Plasma Lactate Assayed by Comparison with Capillary Gas Chromatography, Klaiza E. Brooks1 and Norman B. Smith1,2, * 1 Dept. of Clin. Biochem., Univ. Hosp. Campus, London Health Sci. Centre, P.O. Box 5339, London, Ontario, Canada N6A 5A5 (address for correspondence); 2 Dept. of Biochem., Univ. of Western Ontario, London, Ontario, Canada; * author for correspondence: fax 519-663-3858; e-mail nsmith@ulian.uwo.ca]

During an instrument consolidation process in our laboratory, we needed to identify our best analyzer for doing plasma lactate assays. However, a comparison of our aac V (Dupont Medical Products, Mississauga, Ont., Canada), Synchron CX5 (Beckman Instruments, Mississauga, Ont., Canada), TDx (Abbott Diag-
nostics, Mississauga, Ont., Canada), and Ektachem 700 (John-
son & Johnson, Toronto, Ont., Canada) showed some disagree-
ment in the results. We therefore conducted a study to assess the 
results from the four different instruments, using a capillary 
gas-chromatographic procedure as an independent comparative 
method. All protocols complied with the standards of the ethics 
committee of this hospital, and further details of the methods 
outlined below may be obtained from the authors upon request.

For the comparison, we analyzed 50–55 patients' samples for 
which a routine lactate estimation had been requested. Most of 
these patients were in intensive care, but no attempt was made to 
select samples according to patient history. These samples were 
collected and analyzed over ~2.5 months, which included days in 
in which no testing was done because of holidays (e.g.) or 
unavailability of the gas chromatograph because of competition 
for instrument time for other tests. All testing was done during 
the day shift, usually within a 1-h period but not more than 2 h.

Up to the time of this study, all lactate tests in our laboratory 
had routinely been performed singly with the aca V. This 
routine was maintained during the study, but the results from 
these single determinations were used only for clinical purposes 
and not for the correlation study, except to identify which 
samples to analyze. These samples were then determined in 
duplicate by each of the four instruments being assessed. The 
gas-chromatographic analyses were also done in duplicate, and 
of all of them were performed by one person. The butyl 
 derivatives of the plasma extracts were quantified with a Hewlett-Packard 
Canada (Mississauga, Ont., Canada) Model 5880 gas chromato-
graph equipped with a 30 m Supelco Canada (Mississauga, Ont., 
Canada) methylsilicone capillary column. This method, like 
other gas-chromatographic methods, detects and measures total 
lactate (both D- and L-isomers) as a single peak. However, the 
appearance of D-lactate in plasma is sufficiently rare [1] that 
none of the samples in this study was likely to contain measurable 
amounts of it.

During the second half of the study, we tried to select samples 
on the basis of the initial aca V results, to fill in gaps in the 
concentration range studied as much as possible, so that the 
regression analyses could be performed on reasonably evenly 
distributed data and thus would be as reliable as possible. No 
other selection process was used in this study.

Regression analyses on the means of duplicate lactate deter-
minations comparing each test method with the gas-chromato-
graphic method showed linearity for all test instruments except 
the Ektachem 700; the best fit of the regression plot of the 
Ektachem 700 was quadratic. The data were in the range of 
0.5–3.2 mmol/L, which includes the entire plasma lactate ref-
ter interval (0.5–2.0 mmol/L). We accumulated a few data at 
concentrations >3.2 mmol/L, up to 12 mmol/L, but did not 
include them in these analyses, because the large gaps among 
these data would have yielded results with inordinate weighting 
being given to the more-isolated data points at high lactate 
concentrations. The equations of best fit derived from these 
alyses are given in Table 1. The standard deviations of the 
linear regressions (Table 1) were in increasing order: aca V > 
TDx > Synchron CX5.

Figure 1 shows the bias plots [2] for each test method vs the 
gas-chromatographic method. Because the slopes of the plots 
for the Synchron CX5, TDx, and aca V were not significantly 
different from 0, we calculated the mean biases for these 
methods (Table 1). For the Synchron CX5 the mean bias was 
slightly positive (P <0.001); for the TDx it was not significantly 
different from 0; and for the aca V it was negative (P <0.001). 
The 95% confidence intervals for the mean bias of the Synchron 
CX5 were noticeably smaller than those of TDx and aca V. The 
Ektachem 700, however, appeared to be the most precise 
instrument. On the other hand, the bias for the Ektachem 700 
decreased nonlinearly with increasing lactate concentration 
(0.5–3.2 mmol/L) from a bias of ~0.5 mmol/L to a small 
positive value (~0.062 mmol/L) at a concentration of 2.4 
mmol/L, just above the upper reference limit for lactate.

Eight patients' samples in the correlation study were icteric. 
We analyzed the data in the study both including and excluding 
these samples. Because including them had little effect on the 
results and no effect on the conclusions, we kept them in the 
study.

The results of this study comparing the performances of the 
methods showed that each method exhibited unique, statistically 
distinct, operational characteristics. For given comparisons of 
data between one of the test methods and the gas chromatog-
raphy, any scatter of data would be a combined effect originating 
both from the test method and the gas-chromatographic 
method, whereas differences in the magnitude of the scatter 
among the comparisons would be due solely to the test method 
because the gas chromatography data would be common to all of 
them. Any biases would originate from the test method.

Although the four test methods displayed statistical differences 
in bias and precision, these differences may not be 
clinically significant. Panteghini and Pagani showed that, at a 
mean plasma lactate concentration of 0.64 mmol/L, the analy-

<p>| Table 1. Summary of the performance data derived from regression analyses and bias plots comparing the test methods (y) with the gas-chromatographic method (x). |
|---|---|---|---|---|</p>
<table>
<thead>
<tr>
<th>n</th>
<th>Synchron CX5</th>
<th>TDx</th>
<th>aca V</th>
<th>Ektachem 700</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression equation</td>
<td>y = 1.036x + 0.025</td>
<td>y = 0.990x + 0.020</td>
<td>y = 1.062x - 0.299</td>
<td>y = 0.113x² + 0.462x + 0.702</td>
</tr>
<tr>
<td>Sx,y</td>
<td>±0.122</td>
<td>±0.178</td>
<td>±0.229</td>
<td>-</td>
</tr>
<tr>
<td>Bias equation</td>
<td>y = 0.0906 ± 0.121</td>
<td>-0.0157 ± 0.168</td>
<td>-0.162 ± 0.249</td>
<td>-</td>
</tr>
<tr>
<td>Mean bias ± SD</td>
<td>±0.0363 ± 0.0505</td>
<td>±0.0730</td>
<td>±0.043b</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All units are mmol/L. CI, confidence interval.

a See curve in Fig. 1.

b Estimated from Fig. 1 for a bias of +0.062 mmol/L at a lactate concentration of 2.4 mmol/L.
Fig. 1. Bias plots of plasma lactate concentrations measured by the test methods vs those of the gas-chromatographic method. The solid curve through each set of data represents the line of best fit by linear or nonlinear regression. The dashed curves represent the 95% confidence intervals of the regression lines. O, icteric samples.

The goal for imprecision (CV) was 13.6% [3]. Thus performances of all of the methods may be satisfactory. However, as Fraser and Peterson have argued, even when biological variation, not analytical variation, is the limiting source of variation in a test, there is still great advantage in using a method with the least possible imprecision and bias [4].

The aca V was the least precise instrument and had the largest mean bias. The overall performances of the other methods were close to each other, although each had its own characteristics. The Ektachem 700 appeared to be the most precise instrument, but its positive bias was significant throughout the lactate reference interval, approaching zero just above the upper limit.

In our view, these bias characteristics are less than desirable; others might disagree. The TDx exhibited no bias, but the imprecision was noticeably greater than that of both the Ektachem 700 and the Synchron CX5. Besides having an acceptable imprecision, the Synchron CX5 had only a slightly positive bias.

The performances of all instruments arguably were clinically acceptable and, as is evident, no one method proved to be clearly superior to all others. However, each laboratory must make its selection based on criteria appropriate to its own needs. For our laboratory, we concluded that the Synchron CX5 was the most suitable for measuring lactate in plasma.

References

Influence of Hemoglobin S Adducts on Hemoglobin A, Quantification by HPLC, David D. Sub, Jonathan S. Krauss,* and Keith Bures (Med. College of Georgia, Dept. of Pathol., BIH 222B, 1120 15th St., Augusta, GA 30912-3620; *author for correspondence: fax 706-721-7837, e-mail jkrauss@mail.mcg.edu)

Increased hemoglobin A, (HbA,) is an important finding in the diagnosis of β-thalassemia [1]. In the presence of a β-chain variant hemoglobin such as HbC or HbS, HbA, is known to be slightly increased [2]; Whitten and Rucknagel [3] suggested that this increase reflected impaired association of β-S with α-chains,