Is Prostate-Specific Antigen Present in Female Serum?

To the Editor:

Prostate-specific antigen (PSA) is a tumor marker widely used for the diagnosis and management of patients with prostate cancer. Despite the original notion that PSA was a prostatic tissue-specific marker, it is now well accepted that PSA can be found in many nonprostatic tissues and fluids *[1]*. With the advent of highly sensitive methods for measuring trace amounts of PSA, it became possible to show that female sera demonstrate PSA immunoreactivity *[2–4]*. If PSA immunoassays with detection limits of ~1 ng/L are used, then ~50% of female sera are positive for PSA. However, because of very low concentrations of PSA in female serum, it is impossible to characterize the immunoreactivity in detail and prove that it indeed represents PSA and not immunoassay noise (cross-reactivity or nonspecific effects) *[5]*. Here, we describe a method that demonstrates that the immunoreactivity in female serum is not due to nonspecific effects of the immunoassay used.

We first developed a method that is capable of recognizing purified seminal plasma PSA down to 0.25 ng/L. This assay is essentially identical to the one described by us previously *[6]*, but we replaced the final time-resolved fluorometric measurement of alkaline phosphatase with a chemiluminescence detection method using the substrate CDP-Star™ from Tropix, Inc. Substrate incubation was for 15 min at room temperature. The monoclonal antibodies used in this assay are the same as in our previous assays *[6]*. This PSA assay uses a mouse monoclonal capture antibody encoded 8301 and a biotinylated mouse monoclonal detection antibody encoded 8311 (both from Diagnostic Systems Laboratories). For nonspecificity studies, we used another mouse monoclonal antibody—against α-fetoprotein (AFP)—from the same manufacturer.

The detection limit of this assay, defined as the concentration of PSA that corresponds to the signal of the zero calibrator plus 2 SD, was 0.25 ng/L. Currently, no commercial assay measures PSA concentrations <10 ng/L.

To study whether the PSA immunoreactivity in female serum is indeed specific, we followed this method. We selected 12 female sera that were tested by the PSA assay reported previously *[6]* and had immunoreactivity of 0–550 ng/L. We then prepared two 400-μL aliquots per sample. To the first aliquot we added 1 μL (1 μg) of the 8301 PSA antibody, and to the second aliquot we added 1 μL (1 μg) of the AFP antibody. We then incubated both aliquots for 4 h at room temperature. All aliquots were assayed in triplicate, and PSA concentrations were determined from the calibration curve (data not shown).

Table 1 summarizes the PSA concentrations in antibody-supplemented female sera. Clearly, PSA immunoreactivity was detected in all sera supplemented with the AFP antibody (control), but it essentially disappeared when the sera were supplemented with the 8301 antibody, which is identical to the immobilized capture antibody. Immunoreactive amounts of unsupplemented sera were identical to the concentrations in the sera to which the anti-AFP antibody was added (data not shown). These data support the view that the measured immunoreactivity is PSA specific, because the matrices of the two aliquots of each sample were essentially identical and contained the same amounts of the added mouse monoclonal antibodies. The PSA immunoreactivity from the one aliquot disappeared because it reacted with the 8301 antibody and thus became incapable of binding to the same antibody in the solid phase.

Table 1. PSA concentrations in antibody-supplemented female sera.

Female serum	PSA,[a] ng/L, in serum supplemented with Anti-AFP (control)	Anti-PSA
1	0.27	ND[b]
2	1.26	ND
3	4.22	ND
4	8.83	0.28
5	9.50	ND
6	10.87	ND
7	11.05	ND
8	15.94	ND
9	17.17	ND
10	17.20	ND
11	536.96	ND
12	554.11	ND

[a] PSA not detected: calculated according to the calibration curve. All samples were run in triplicate.
[b] ND, PSA <0.25 ng/L (the detection limit of the assay).

These data support the view that PSA is a normal constituent of female serum. Possible diagnostic applications of PSA measurements in female serum have been proposed recently *[7–10]*.

References

1. Diamandis EP, Yu H. New biological functions of prostate-specific antigen? J Clin Endocrinol Metab 1995;80:1515–7.
2. Diamandis EP, Yu H, Melegos DN. Ultrasensitive prostate-specific antigen assays and their clinical application. Clin Chem 1996; 42:853–7.
3. Yu H, Diamandis EP. Measurement of serum prostate specific antigen levels in females and in prostatectomized males with an ultrasensitive immunoassay technique. J Urol 1995;153:1004–8.
4. Giai M, Yu H, Roagna R, Ponzone R, Katsaros D, Levesque MA, Diamandis EP. Prostate specific antigen in serum of women with breast cancer. Br J Cancer 1995;72:728–31.
5. Stamey TA. Lower limits of detection, biological detection limits, functional sensitivity, or residual cancer detection limit? Sensitivity reports on prostate-specific antigen assays mislead clinicians. Clin Chem 1996;42: 849–52.
6. Ferguson RA, Yu H, Kalyvas M, Zammit S, Diamandis EP. Ultrasensitive detection of prostate-specific antigen by a new time-resolved immunofluorometric assay and the Immulite immunochemiluminescent third-generation assay: potential applications in prostate and breast cancers. Clin Chem 1996;42:675–84.
7. Melegos DN, Yu H, Allen LC, Diamandis EP. Prostate specific antigen in amniotic fluid of normal and abnormal pregnancies. Clin Biochem 1996;29:555–62.
8. Zarghami N, Grass L, Sauter ER, Diamandis EP. Prostate-specific antigen levels in serum during the menstrual cycle. Clin Chem 1997; 43:1862–7.
9. Melegos DN, Yu H, Ashok M, Wang C, Stanczyk F, Diamandis EP. Prostate specific antigen in female serum—a potential new marker of androgen excess. J Clin Endocrinol Metab 1997;82:777–80.
10. Melegos DN, Diamandis EP. Diagnostic value of molecular forms of prostate specific antigen for female breast cancer. Clin Biochem 1996;29:193–200.

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γ-Hydroxybutyrate Concentrations in Pre- and Postmortem Blood and Urine

To the Editor:

With γ-hydroxybutyrate (GHB) becoming popular as a drug of abuse in the US and elsewhere *[1]*, we are receiving increasing requests for the analytical determination of GHB in blood or urine in criminal investigations, especially in sexual assault cases. In a recent report of a fatal poisoning with GHB, the victim had a postmortem blood GHB concentration of 27 mg/L *[2]*, and another three GHB-related fatalities were reported with postmortem blood GHB concentrations of 52–121 mg/L *[3]*.

As a part of a validation study before instituting a GC-MS method described by others *[4]*, we tested for GHB presence in a series of forensic specimens submitted routinely to us by law enforcement agencies and medical examiner offices in cases not known to be GHB-related. No GHB was detected (detection limit, 1 mg/L) in the blood or urine of living persons or in postmortem urine, but very substantial concentrations, ranging from 3.2 to 168 mg/L, were found in 15 of 20 autopsy blood specimens (Table 1). Reanalysis of these 20 blood specimens by gas chromatography with flame-ionization detection on a packed column *[5]* gave similar qualitative and quantitative results.

These results have great potential significance to the interpretation of postmortem blood GHB concentrations, because the concentration range of this apparent "endogenous" GHB overlaps that known to produce clinical effects in patients receiving the drug as an anesthetic agent *[6]* as well as the concentrations reported to be associated with fatal reactions in medicolegal investigations *[2, 3]*. The fact that substantial GHB concentrations are found in the blood of deceased persons but not in living persons suggests that GHB is a product of postmortem decomposition.

Table 1. GHB concentrations in blood (n = 20) and urine (n = 8) by GC-MS.

	GHB concn., mg/L	
Specimen	**Ave.**	**Range**
Living persons		
Blood	0	0–0
Urine	0	0–0
Deceased persons		
Blood	25	0–168
Urine	0	0–0

We are unable to state from our data whether factors such as environmental temperature or storage time contribute to an increase in postmortem blood GHB concentrations. However, we suggest that analysis of GHB in urine, in which the concentrations tend to parallel those in blood but are ~10-fold greater *[6]*, would produce more meaningful results in the investigation of drug-related death.

References

1. Centers for Disease Control. Multistate outbreak of poisonings associated with illicit use of gamma hydroxy butyrate. JAMA 1991;265: 447–8.
2. Mozayani A, Brown T, Carter J. The tip of the iceberg in the Harris County Forensic Center. Presentation at Am Acad Forensic Sci annual meeting, New York, NY, February 20, 1997.
3. Woodward W, Todd E. Gamma-hydroxybutyrate. Presentation at Southwestern Assoc Toxicol semiannual meeting, San Antonio, TX, April 5, 1997.
4. Ferrara SD, Tedeschi L, Frison G, et al. Therapeutic gamma-hydroxybutyric acid monitoring in plasma and urine by gas chromatography–mass spectrometry. J Pharm Biomed Anal 1993;11:483–7.
5. van der Pol W, van der Kleijn E, Lauw M. Gas-chromatographic determination and pharmacokinetics of 4-hydroxybutyrate in dog and mouse. J Pharmacokinet Biopharm 1995;3: 99–113.
6. Baselt RC, Cravey RH. Disposition of toxic drugs and chemicals in man, 4th ed. Foster City, CA: Chemical Toxicology Institute, 1995: 348–9.

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Joint Limiting Values of N, Mean, and SD

To the Editor:

A graphical Win32 computer program for variance function estimation *[1]* is currently under development in this department, and design of the data entry module produced an interesting "impossible values" issue. The program incorporates two distinct data entry spreadsheets: (*a*) a grid for entering or importing sets of raw replicated measurements (e.g., runs of QC or other precision results), and (*b*) a grid for entering sets of values of N, mean, and SD (e.g., to allow estimation of a variance function from the summarized precision data often found in the literature). Restricting raw measurements to a sensible laboratory oriented range (e.g., $0–10^7$) is a simple matter, but constraining values of N, mean, and SD was more problematic. Suppose, for example, a user enters N = 10, mean = 1.0, and SD = 10 000; each value is within numerical limits, but clearly there is no distribution of 10 values (≥0) that can simultaneously have the mean = 1.0 and SD = 10 000. Allowing the manipulation of impossible values is unsatisfying and might also cause numerical instability or even failures if users experiment with highly extreme combinations.

The limiting relationship is surprisingly simple; the largest observable SD for any set of N values (≥0) is

$$SD_{max} = \sqrt{N} \times \text{mean}.$$

Thus, in addition to initial data entry constraints (e.g., N ≥2, mean and SD ≥0, and all values less than a defined upper limit), the following rules can