Forensic Science Communications April 2003 — Volume 5 — Number 2

Research and Technology

Estimation of Prostate-Specific Antigen (PSA) Extraction Efficiency from Forensic Samples Using the Seratecâ PSA Semiquant Semiquantitative Membrane Test

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Introduction

Prostate-specific antigen (PSA, also known as p30) is a glycoprotein produced by the prostate gland that has been well characterized and validated in the forensic science community as a marker for the presence of seminal fluid (Hochmeister et al. 1999; Johnson and Kotowski 1993; Poyntz and Martin 1984; Sensabaugh 1978). The Seratecâ PSA Semiquant (Seratecâ Diagnostica, Göttingen, Germany) membrane, and the *OneStep ABA card*â p30 (Abacus Diagnostics, West Hills, California) are immunochromatographic membrane tests that allow for rapid, convenient, and highly sensitive analysis for the presence of seminal fluid (Hochmeister et al. 1999). The Seratecâ PSA Semiquant test membrane also offers a semiquantitative test for prostate-specific antigen by the use of an internal standard. A visible line, roughly equivalent in intensity to a concentration of 4ng PSA/ml is available on the test strip allowing a visual comparison with the test line concentration (Seratecâ Diagnostica 2000).

Methods and Materials

In this study, liquid semen sample dilutions were identified as being near the 4ng PSA/ml internal standard value. Forensic semen stains and swabs were then prepared with known quantities of these semiquantified semen samples. Semen from both a vasectomized individual and a nonvasectomized individual were serially diluted to 1:1,000,000 with sterile water. Swabs were prepared from the dilutions using approximately 150µl of solution per swab by allowing the swab to saturate and then to air-dry. Stains (approximately 44.5mm diameter, approximately 1555mm^2 total area) were also prepared on clean cotton cloth using 500µl of the semen dilutions and then allowed to air-dry.

Duplicate extractions of approximately 1/3 of each swab (vasectomized semen only) were performed in 250µl of sterile water for two hours at room temperature. After centrifugation for one minute at 13,000g, 200µl of the supernatant was

removed and tested with the Seratecâ PSA Semiquant membrane (x2) and the *OneStep ABA card*â p30 (x1).

Duplicate extractions of approximately 5mm square cuttings (25mm^2) from the center of the prepared semen stains (nonvasectomized semen only) on cotton fabric were performed in 250µl of sterile water for two hours at room temperature. After centrifugation for one minute at 13,000g, 200µl of the supernatant was removed and tested with the Seratecâ PSA Semiquant membrane (x2) and the *OneStep ABA card*â p30 (x1).

Duplicate extractions of approximately 5mm square cuttings (25mm^2) from the center of the prepared semen stains (nonvasectomized semen only) on cotton fabric were performed in 250µl of 1X HEPES buffer (137.0mM NaCl, 5.0mM K Cl, 0.8mM Na2HPO4-2H2O, 5.5mM dextrose, 21.0mM HEPES) for two hours at room temperature. After centrifugation for one minute at 13,000g, 200µl of the supernatant was removed and tested with the Seratecâ PSA Semiquant membrane (x2).

Two hundred microliters of the prepared liquid semen dilutions were tested with the Seratecâ PSA Semiquant membrane (x1) and the *OneStep ABA card*â p30 (x1).

Results

Estimation of Semen PSA Concentration

The evaluation of both the vasectomized and the nonvasectomized liquid semen dilutions using the Seratecâ PSA Semiquant membrane indicated that the concentration at 1:400,000 was greater than the 4ng PSA/ml internal standard marker and that the concentration at 1:500,000 was less than the 4ng PSA/ml internal standard marker. The 4ng PSA/ml internal standard equivalency point was estimated at 1:450,000.

This estimation was corroborated by the *OneStep ABA card*â p30 results. The *OneStep ABA card*â p30 has a published sensitivity of 4ng PSA/ml. The largest dilution that gave a positive result with the nonvasectomized semen was 1:400,000. The largest dilution that gave a positive result with the vasectomized semen using the *OneStep ABA card*â p30 membrane test was 1:500,000. The estimated dilution that is roughly equivalent to 4ng PSA/ml (1:450,000) implies that the whole semen samples contain approximately 1,800,000ng PSA/ml, well within the published normal range (Abacus Diagnostics 2001; Seratecâ Diagnostica 2000).

A PSA standard (Sigma-Aldrich catalog number 3338) was diluted with 0.15M phosphate buffered saline (Sigma-Aldrich catalog number P3813) to concentrations from 8ng/ml to 2ng/ml and analyzed with the Seratecâ PSA Semiquant membrane to assess the accuracy of the semiquant assay. The ability of the assay to discriminate between these low concentrations of prostate-specific antigen was assessed and found to be satisfactory for the purposes of estimating extraction efficiencies. The accuracy of the semiquant line was within a factor of two of the reported approximation of 4ng PSA/ml (data not shown).

Estimation of Extraction Efficiencies

Results using the Seratecâ PSA Semiquant membrane and the *OneStep ABA card*â p30 were noted after ten minutes as either positive ([+] with control and test lines present) or negative ([-] with control line present and test line absent) using the individual manufacturer's instructions (Abacus Diagnostics 2001; Seratecâ Diagnostica 2000).

Positive test lines that were identified with the Seratecâ PSA Semiquant membranes were further evaluated for their intensity in comparison to the 4ng/ml semiquant line. The test line was noted as being more intense (T > q), equally as intense (T = Q), or less intense (t < Q) than the 4ng PSA/ml semiquant line.

Results of the liquid semen dilutions are shown in Table 1.

Results of the semen-stained swabs are shown in Table 2.

The PSA concentration of the undiluted semen was estimated at approximately 1,800,000ng PSA/ml; therefore, the total available PSA per prepared swab can be estimated at 0.15ml x 1,800,000ng/ml = 270,000ng/swab. Using 1/3 of a swab and a 0.250ml extraction volume, the total available PSA concentration becomes approximately 360,000ng/ml. At 100 percent extraction efficiency, a 1:90,000 dilution could be expected to have a PSA concentration of approximately 4ng/ml.

The evaluation of the extractions from the swabs indicated that the 4ng PSA/ml internal standard was roughly equivalent to the results obtained with the swab prepared with a 1:100 semen dilution. This indicates that approximately 4ng/ml of the available 360,000ng/ml PSA was extracted, implying a 1/900, or approximately 0.11 percent, PSA extraction efficiency from these swabs.

Results of the semen stains on cotton cloth are shown in Table 3.

At the approximately 1,800,000ngPSA/ml undiluted semen concentration estimated above, the total available PSA per prepared ~ 44.5mm diameter stain can be estimated at 0.5ml x 1,800,000ng/ml = 900,000ng/stain. Using approximately 1/62 of the stains (25 mm^2 / ~1555 mm^2), and a 0.250ml extraction volume, the total available PSA concentration becomes approximately 58,000ng/ml. At a 100 percent extraction efficiency, a 1:14,500 dilution could be expected to have a PSA concentration of approximately 4ng/ml.

The evaluation of the extractions from the stains extracted in sterile water indicated that the 4ng PSA/ml internal standard was roughly equivalent to the results obtained with the stain prepared with a 1:50 semen dilution. This implies an approximately 1/290, or 0.34 percent, PSA extraction efficiency from these prepared stains.

The evaluation of the extractions from the stains extracted in 1X HEPES buffer indicated that the dilution that was closest to the 4ng PSA/ml internal standard was the stain prepared with a 1:100 semen dilution. At this dilution the 4ng/ml standard line was slightly less intense that the test line, hence an estimate of a 1:150 semen dilution for equivalence to the semiquant line was used. This estimation would imply an approximately 1/97, or 1.03 percent, PSA extraction efficiency from these prepared stains.

It was noted that positive results were not obtained at lower concentrations when using the HEPES buffer despite the three-fold higher estimate of PSA extraction efficiency over extractions using sterile water at the 4ng PSA/ml equivalence level.

Conclusions

Comparison of the stain and swab extracts with the liquid semen sensitivity results shows that it can be estimated that one percent or less of the PSA applied to the swabs and stains was able to be extracted. This estimation of PSA extraction efficiency indicates that the yield from common forensic substrates is one percent or less of the estimated total available PSA. Whereas the sensitivity of immunochromatographic cassettes using diluted liquid semen samples is quite

high, the PSA extraction efficiency when analyzing liquid extracts from dried semen dilutions is low enough to limit their usefulness. This study shows that a more efficient extraction process would be beneficial when applying these types of membrane tests to forensic science applications.

If these extraction efficiency estimations are also valid for prostate-specific antigen found in adult male postejaculate urine and blood serum, then concerns regarding positive test results from these sources may be undeserved. Liquid postejaculate urine samples are reported to have a mean value of 260ng PSA/ml, and this must be considered when analyzing liquid samples (Abacus Diagnostics 2001; Seratecâ Diagnostica 2000). However, if dried samples are submitted and extraction efficiencies are one percent or less, these same samples would yield less than 2.6ng PSA/ml. This PSA concentration is much less of a concern as a possible confounding factor during evidence examination because it is less than the stated sensitivity of the *OneStep ABA cardâ* p30 and less than the semiquant line of the Seratecâ PSA Semiquant membrane.

References

Abacus Diagnostics. *OneStep ABA Cardâ*. Abacus Diagnostics, West Hills, California, 2001.

Hochmeister, M. N., Borer, U., Budowle, B., Dirnhofer, R., Gehrig, C., Rudin, O., and Thali, M. Evaluation of prostate-specific antigen (PSA) membrane test assays for the forensic identification of seminal fluid, *Journal of Forensic Sciences* (1999) 44:1057-1060.

Johnson E. D. and Kotowski T. M. Detection of prostate-specific antigen by ELISA, *Journal of Forensic Sciences* (1993) 38:250-258.

Poyntz, F. M. and Martin, P. D. Comparison of PSA and acid phosphatase levels in postcoital vaginal swabs from donor and casework studies, *Forensic Science International* (1984) 24:17-25.

Sensabaugh G. Isolation and characterization of a semen-specific protein from human seminal plasma: A potential new marker for semen identification, *Journal of Forensic Sciences* (1978) 23:106-15.

Seratecâ Diagnostica. *Seratecâ PSA Semiquant Membrane Test for Detection of Seminal Fluid*. Seratecâ Diagnostica, Göttingen, Germany, 2000.

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