TECHNICAL NOTE

John P. Simich,[1] Ph.D.; Shannon L. Morris,[2] M.S.; Robert L. Klick,[2] M.S.; and Kate Rittenhouse-Diakun,[2] Ph.D.

Validation of the Use of a Commercially Available Kit for the Identification of Prostate Specific Antigen (PSA) in Semen Stains

REFERENCE: Simich JP, Morris SL, Klick RL, Rittenhouse-Diakun K. Validation of the use of a commercially available kit for the identification of prostate specific antigen (PSA) in semen stains. J Forensic Sci 1999;44(6):1229–1231.

ABSTRACT: PSA is currently being used to detect and monitor quantitatively the development of prostate cancer by serum levels of PSA and has also been found to be present in high concentrations in semen. Elegantly simple, sensitive, and reproducible methods have been developed for analysis of the presence of PSA, including the Tandem-E PSA Immunoenzymetric Assay. The most common procedures for the forensic identification of semen have focused on the microscopic detection of sperm, acid phosphatase activity, and immunoelectrophoretic methods for the detection of PSA. Although these methods have been used for many years, there are problems associated with each method. The Tandem-E PSA Immunoenzymetric Assay detected PSA in 100% of the forensic casework fabric samples, 80% of the forensic casework vaginal swabs and 100% of the vasectomized individuals tested. The cut-off value was determined to be 1.77 ng/mL. These results indicate that this method can be used to identify the presence of semen in forensically significant specimens.

KEYWORDS: forensic science, prostate specific antigen, P30, semen stains, enzyme linked immunosorbent assay, sexual assault, validation, assay

In the United States, a majority of the cases in forensic serology labs involve alleged rape and sexual assaults. Ideally, to say conclusively that ejaculation has occurred, spermatozoa must be detected in the semen isolated from the victim. However, under some circumstances, such as vasectomy, sperm may be absent from the semen.

In the absence of spermatozoa, other tests such as the acid phosphatase test or the PSA assay can be used to detect the presence of semen (1–4). Prostate specific antigen is a serine protease produced by prostatic epithelial cells and is found in seminal fluid, prostatic fluid, male serum and male urine (1,5,6). A study by Graves et al. found that PSA possesses many characteristics of an ideal marker for the detection of semen in the investigation of sexual assault (1). The mean vaginal decay time of PSA was found to be 27 h, while acid phosphatase decline from the same postcoital vaginal swabs showed a mean vaginal decay time of 14 h (1). Low levels of PSA have also been found in apocrine sweat glands, in the breast milk of lactating women and in some breast tumors (7–9). Even though PSA is not gender and tissue specific, in sexual assault cases, this should not pose a significant problem because of the very low concentrations of PSA in nonprostatic fluids (10).

Since 1986, when PSA was approved by the FDA for use in monitoring prostate cancer, and in 1994 as a screening marker, many different methods of analysis for PSA have been developed. An ELISA based commercial test kit for detecting PSA in serum samples is currently manufactured by Hybritech (Beckman Instruments). The Hybritech Tandem-E PSA Immunoenzymetric Assay is used in the clinical setting for the quantitation of PSA in the serum of males at risk for prostate cancer (11).

The purpose of this study was to validate the use of the Hybritech Tandem-E PSA Immunoenzymetric Assay to detect PSA in forensic casework samples. This method was evaluated for its sensitivity and specificity, and compared to other conventional methods of semen identification on numerous adjudicated casework samples.

Materials and Methods

Fabric and Swab Stain Extraction

All fabric and swab samples were obtained from casework samples or as indicated below and stored at –22°C following collection until analyzed. Additionally, negative fabric controls were collected when applicable. For fabric stain and control extractions, a 0.5 cm × 0.5 cm area was cut from the fabric and put into a 1.5 mL microcentrifuge tube. For swab extractions, the whole cotton head of the swab was removed and put into a 1.5 mL microcentrifuge tube. One mL of PBS was added to each tube. The tubes were briefly vortexed, incubated at room temperature for 30 min, followed by exposure to ultrasound for 5 s. The fabric cuttings or swabs were removed and the tubes were centrifuged at 10,000 rpm for 3 min. The supernatant was removed to a clean tube and frozen. The pellet was applied to a microscope slide, air-dried and the slides were stained with nuclear fast red/picroindigocarmine

[1] Senior forensic serologist, Erie County Central Police Services, Forensic Laboratory.

[2] Former graduate student, associate professor, and assistant professor, respectively, Department of Clinical Laboratory Science, State University of New York at Buffalo.

Received 13 April 1998; and in revised form 8 Sept. 1998, 12 Feb. 1999; accepted 15 Feb. 1999.

stain and examined microscopically for the presence of spermatozoa.

Qualitative Acid Phosphatase Test

The extracted samples were tested for the presence of acid phosphatase using a modification of a previously published protocol (12) using α-naphthyl phosphate as the substrate.

Hybritech Tandem-E PSA Immunoenzymetric Assay

The assay was performed according to the procedure described in the Hybritech Tandem-E PSA procedure manual (11) and as follows. The recommended sample volume of 100 μL of the zero diluent, calibrators, controls, and samples was used. Casework samples were diluted and re-assayed as required to obtain an on scale PSA value (<100 ng/mL). All calibrators and controls were run in duplicate, and all test samples were run in triplicate.

Evaluation of Negative Vaginal Swabs

Vaginal swabs were collected from ten women in accordance with approved guidelines for the protection of human subjects. All women abstained from sexual intercourse for a minimum of five days prior to swab collection. Each subject was given two sterile cotton swabs and instructed to insert them one at a time one inch into the vaginal canal for 15 s. The swabs were air-dried, returned to the laboratory and frozen until use. One swab from each donor was examined as described above.

Evaluation of Cadaver Swabs

Samples were provided by the Erie County Medical Examiner's Office. The samples were randomly collected from 11 female cadavers. A swab was inserted into the vaginal canal of each subject, removed and frozen until use. Each swab was examined as described above.

Vasectomized Semen Samples

Three seminal fluid samples from vasectomized individuals were obtained from a physician's office. The samples were examined as described above.

Saliva Samples

Saliva samples were obtained from one male and one female volunteer and frozen until use. For analysis, the samples were thawed and analyzed for PSA using the Hybritech Tandem-E PSA Test Kit.

Blood Contamination Study

A fabric sample containing a semen stain was mixed with blood (in approximately a 1:2 ratio) from three different volunteers, two males and one female. The fabric was air dried and extracted as previously described. The samples were examined as above.

Linearity Study

A seminal fluid standard (SERI) was prepared to yield a starting value of 50 ng/mL (neat) and was analyzed in duplicate for PSA at the following dilutions: neat, 1/10, 1/100, 1/500, 1/1000.

Species Specificity Study

Pure semen samples from six primates and one dog were collected and frozen until use. The samples were examined as described above.

Results and Discussion

Fourteen fabric samples with stains and twelve fabric control areas consisting of denim, cotton and cotton blends were analyzed for acid phosphatase activity, spermatozoa and PSA. While spermatozoa were identified in all 14 samples, acid phosphatase was positive in thirteen. Values of PSA ranged from a low of 30.44 ng/mL to a high of 16,420 ng/mL, with a mean of 1969 ng/mL, and a median of 417 ng/mL.

All fabric control areas were negative for acid phosphatase and spermatozoa. The values obtained for PSA ranged from 0.000 ng/mL to 1.311 ng/mL of PSA, with a mean of 0.2 ng/mL, and a median of 0.07 ng/mL. Statistical analysis indicates that the PSA values obtained for the fabric stains versus fabric controls are statistically different ($p = 0.0011$). Likewise, comparison of the casework vaginal swabs ($p = 0.005$) with the negative control vaginal swabs and the cadaver swabs ($p = 0.005$) demonstrated a statistical difference.

A cut-off value for specimen positivity was determined by calculating the least detectable dose (LDD) for negative fabric controls. The LDD was determined by taking the mean value of PSA for negative samples plus four times the standard deviation. Using this criteria, the resulting cut-off value for negative samples was determined to be 1.77 ng/mL with a 99.997% certainty that no false positives will occur. The least detectable doses for negative vaginal swabs and cadaver swabs were determined to be 0.87 ng/mL and 0.21 ng/mL of PSA, respectively.

Semen samples from three vasectomized individuals were examined for acid phosphatase, spermatozoa, and PSA. All samples were negative for spermatozoa and were positive for PSA with a range from 109.6 ng/mL to greater than 50,000 ng/mL.

Semen was obtained from seven different animals (six apes, one canine). All samples were positive for spermatozoa, while only four were positive for acid phosphatase activity. All animal semen samples were positive for PSA with values ranging from 28.68 ng/mL to >200,000 ng/mL (Table 1).

Body fluid contaminants were evaluated to determine if these fluids would produce false positive PSA results. Saliva samples from one female donor and one male donor yielded PSA values of 0.167 ng/mL and 0.115 ng/mL, respectively. Specimen PSA results were below the least detectable dose of the assay. Testing of semen mixed with blood resulted in the recovery of PSA values within the range expected for the control semen specimen, hence no false negative results due to heme inhibition were observed.

A semen standard (SERI R563) was diluted and the observed concentrations of PSA were compared to the calculated values. As shown in Table 2, the acceptable assay linearity was determined to be 0.26 ng/mL to 55.9 ng/mL ($r = 0.994$), using regression analysis. This value is consistent with previously published reports (1,13).

Clearly, the PSA assay correctly classifies specimens positive for semen more accurately than does the seminal acid phosphatase assay. While undiluted semen does exhibit milligram levels of PSA, many casework samples will contain much lower levels due to a loss of PSA (1). There is routinely a delay in the time between when a sexual assault occurs and the samples are collected. Thus an assay capable of detecting nanogram amounts of PSA will allow the laboratory to identify positive semen cases with greater accuracy.

TABLE 1—*Results of acid phosphatase activity, spermatozoa search, and PSA detection for species semen samples.*

Sample Number	AP Activity*	Spermatozoan**	PSA ng/mL***
Pygmy Chimp.	+ str.	+ + + +	163.2
Drill	−	+ + + +	77.4
Chimp.	+ str.	+ + + +	> 200,000
Gorilla	−	+ + + +	28.68
Orangutan	+ str.	+ + + +	> 200,000
Chimp.	+ str.	+ + + +	4013.1
Dog	−	+ + + +	44.7

* + (positive), − (negative) for seminal AP.
** − (negative). + (rare). + + (few). + + + (moderate). + + + + (many).
*** Mean of samples run in triplicate.

TABLE 2—*Results from linearity study.*

Point Number	Observed conc. PSA ng/mL	Calculated conc. PSA ng/mL
1 (neat)	55.976	50
2 (1/10)	5.695	5
3 (1/100)	0.609	0.5
4 (1/500)	0.26	0.1
5 (1/1000)	0.269	0.05

This study demonstrates that the Hybritech Tandem-E PSA Immunoenzymetric Assay can be used to identify the presence of PSA that is of seminal origin in a biological stain. The use of this commercially available kit allows the forensic laboratory to perform a large number of simultaneous analyzes. Thus, this method provides an alternative procedure for semen identification that is less labor intensive and therefore more cost effective than conventional semen identification methods such as sperm identification. Additionally, this kit is available in a microtiter plate format that will allow even more automation and therefore, time saving measures.

Acknowledgments

We thank Dr. Kenneth Gould of the Yerkes Regional Primate Research Center at Emory University for kindly providing the primate semen samples, Dr. David Brummer from the Orchard Park Veterinary Hospital for kindly providing the canine semen sample and the Erie County Medical Examiner's Office for kindly providing the cadaver vaginal swabs.

References

1. Graves HCB, Sensabaugh GF, Blake ET. Postcoital detection of a male-specific semen protein. N Engl J Med 1985;312(6):338–43.
2. Kamenev L, Leclercq M, Francois-Gerard Ch. An enzyme immunoassay for prostate-specific p30 antigen detection in the postcoital vaginal tract. J Forensic Sci Soc 1989;29:233–41.
3. Enos WF, Beyer JC. Prostatic acid phosphatase, aspermia, and alcoholism in rape cases. J Forensic Sci 1980;25(2):353–6.
4. Sensabaugh GF. The quantitative acid phosphatase test. A statistical analysis of endogenous and postcoital acid phosphatase levels in the vagina. J Forensic Sci 1979;24(2):346–65.
5. Sensabaugh GF. Isolation and characterization of a semen-specific protein from human seminal plasma: a potential new marker for semen identification. J Forensic Sci 1978;23(1):106–15.
6. Ban Y, Wang MC, et al. The proteolytic activity of human prostate-specific antigen. Biochem Biophys Res Commum 1984;123:482–8.
7. Papotti M, Paties C, et al. Immunocytochemical detection of prostate-specific antigen (PSA) in skin adnexal and breast tissues and tumors. Basic Appl Histochem 1989;33:25–9.
8. Yu H, Diamandis EF. Prostate-specific antigen in milk of lactating women. Clin Chem 1995;41(1):54–8.
9. Yu H, Diamandis EF, Sutherland DJA. Immunoreactive prostate-specific antigen levels in female and male breast tumors and its association with steroid hormone receptors and patient age. Clin Biochem 1994;27(2):75–9.
10. Graves HCB, Sensabaugh GF, Blake ET. Postcoital detection of a male-specific semen protein: application to the investigation of rape. N Engl J Med 1985;312:338–43.
11. Hybritech. Tandem-E PSA manual. Hyritech,Inc (Beckman Instruments), San Diego, CA 1996;1–5.
12. Biology Methods Manual. Metropolitan Police Forensic Science Laboratory, London, England. 1978;Chapter 3:18.

Additional information and reprints requests:
John P. Simich, Ph.D.
Erie County CPS Forensic Laboratory
74 Franklin St.
Buffalo, NY 14202