

TECHNICAL NOTE

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Evaluation of Prostate-Specific Antigen (PSA) Membrane Test Assays for the Forensic Identification of Seminal Fluid*

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

ABSTRACT: Prostate specific antigen (PSA, also known as p30), a glycoprotein produced by the prostatic gland and secreted into seminal plasma, is a marker used for demonstrating the presence of seminal fluid. Methods for the detection of PSA include Ouchterlony double diffusion, crossover electrophoresis, rocket immunoelectrophoresis, radial immunodiffusion, and ELISA. The extremely sensitive ELISA technique can detect PSA in concentrations as low as approximately 4 ng/mL. However, all these techniques are cumbersome and time consuming to perform in forensic laboratories, especially when only a few samples per week are processed. Various membrane tests are currently used in clinical settings to screen a patient's serum for the presence of PSA at levels greater than 4 ng/mL. In this study we evaluated three immunochromatographic PSA membrane tests by analyzing semen stains stored at room temperature for up to 30 years, post-coital vaginal swabs taken at different time after intercourse, semen-free vaginal swabs, and various female and male body fluids, including urine. The data demonstrate that PSA membrane test assays offer the same sensitivity as ELISA-based tests and provide a rapid approach for the forensic identification of seminal fluid. Furthermore, when the supernatant from a DNA extraction is used for the assay, there is essentially no DNA consumption for determining the presence of PSA in a forensic sample.

KEYWORDS: forensic science, semen, prostate-specific antigen, PSA, p30, confirmatory test, azoospermia, vasectomy

Prostate specific antigen (PSA, also known as p30), a glycoprotein produced by the prostatic gland and secreted into seminal plasma, is a valid marker for detecting semen in evidence from criminal cases including samples deposited by vasectomized or azoospermic individuals (1,2). Methods for the detection of PSA

include Ouchterlony double diffusion, crossover electrophoresis, rocket immunoelectrophoresis, radial immunodiffusion, and ELISA (3–9). However, some of these procedures have low sensitivity and/or are cumbersome and time consuming to perform in forensic laboratories, especially when only a few samples are analyzed per week.

For clinical screening of elevated levels of PSA in serum that may indicate the presence of prostatic cancer (10), several sensitive membrane-based PSA tests have been developed and are commercially available. These tests are simple, relatively rapid to perform, require minimal equipment, and produce results that are easy to interpret. Furthermore, these tests offer the same sensitivity as ELISA-based tests. Thus, PSA membrane tests may also prove useful as confirmatory tests for the presence of seminal fluid in forensic casework analyses. The purpose of this study was to determine whether or not such membrane tests facilitate detection of PSA in routine forensic samples.

Material and Methods

PSA Membrane Test Assays

Three tests: PSA-check-1 (VED-LAB, Parc du Londeau, 61 000 Alencon, France), Seratec[®] PSA Semiquant (Seratec Diagnostica, Gesellschaft fuer Biotechnologie mbH, 337027 Göttingen, Germany); and One Step ABA card PSA (Abacus Diagnostics, 6520 Platt Av. 220, West Hills, CA 91307, USA), with reported sensitivity of detection for PSA as low as 4 ng/mL, were evaluated. All three tests consist of an all inclusive, single test device.

Each device utilizes mobile monoclonal anti-human PSA antibodies, which are conjugated to dye particles and can bind human PSA (Fig. 1). The resulting antigen-antibody complexes migrate on the membrane of the test device to a reaction zone, where immobilized polyclonal antihuman PSA antibodies reside. An antigen-antibody-antigen sandwich concentrates the dye particles, resulting in the formation of a line, indicating the presence of human PSA.

Unbound mobile monoclonal anti-human PSA antibodies migrate on the membrane to a control zone, where immobilized anti-Ig antibodies reside (Fig. 1). A complex is formed, concentrating dye particles and also resulting in the formation of a line. The test is considered valid when one line in the control zone is observed.

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* Names of commercial manufacturers are provided for identification only and inclusion does not imply endorsement by the authors.

Received 10 July 1998; and in revised form 24 Nov. 1998; accepted 25 Nov. 1998.

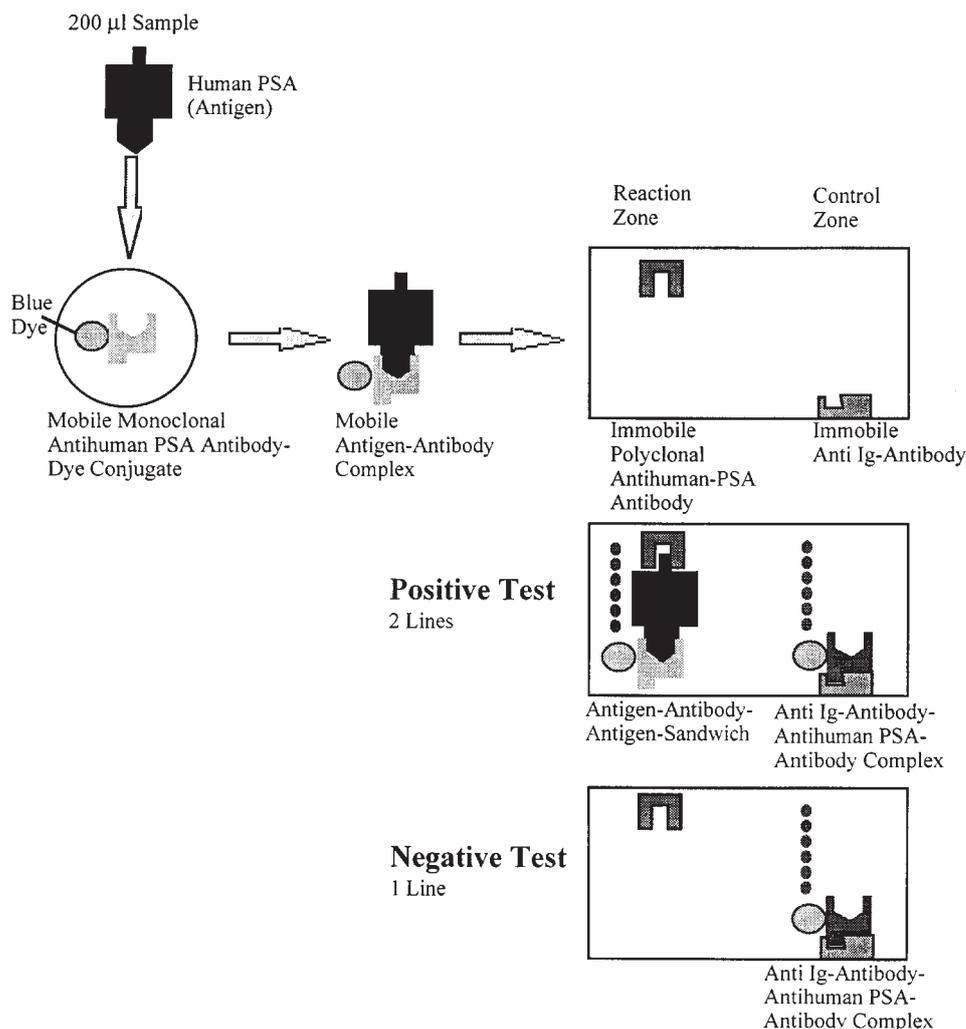


FIG. 1—Unbound mobile monoclonal anti-human PSA antibodies migrate on the membrane to a control zone, where immobilized anti-Ig antibodies reside (Fig. 1).

With a positive test result (>4 ng PSA/mL), two lines appear, with a negative result, only the control line is visible (Figs. 1–3).

From all samples used in this study, 200 µL of an extract were placed in the opening at the end of the test device. The test results were read after 10 min.

Determination of Sensitivity and Specificity

Seminal fluid from 10 non-vasectomized and 10 vasectomized individuals and seminal fluid from cat (*Felis catus*), dog (*Canis familiaris*), pig (*Sus scrofa*), horse (*Equus caballus*), and bull (*Bos taurus*) was serially diluted to 1:10,000,000 with sterile water. Fresh urine samples from 10 males and 10 females were serially diluted to 1:1000 with sterile water.

The microorganisms *Bacillus Clostridium*, *Pseudomonas*, *Escherichia coli*, *Streptococcus*, *Staphylococcus*, and *Candida albicans* were directly removed from the culture media and suspended in 650 µL HEPES buffered saline.

Saliva, perspiration, fecal material, and seminal fluid-free vaginal secretions with and without menstrual blood were recovered on cotton swabs from 10 male and 10 female volunteers. Ten semen stains on cotton cloth stored at room temperature for 2 to 30 years and 50 vaginal swabs from recent sexual assault cases (stored at

room temperature for up to three months) were evaluated for the presence of PSA using these membrane tests. All swabs and stains were extracted in 650 µL of HEPES buffered saline for 2 h at 4°C on a shaker. After centrifugation for 3 min at 13,000 g, 200 µL of the supernatant was removed and serially diluted to 1:10,000. Negative controls (sterile water and HEPES buffered saline) also were prepared and analyzed.

DNA Compatible Assay

All swabs and stains were extracted in 650 µL HEPES buffered saline (11) as described above. After 200 µL of the supernatant were removed for the PSA assay, detergent (sarcosyl) and proteinase K were added to the remaining extract and differential lysis DNA extraction was continued (11).

In the study, an additional 200 µL of supernatant were removed from some samples to verify the absence of DNA in the supernatant using a DNA hybridization assay specific for human DNA (12).

Results and Discussion

The range of PSA is known to be 200,000 to 5.5 million ng/mL of semen (2–5,13). The manufacturers of the PSA membrane-tests

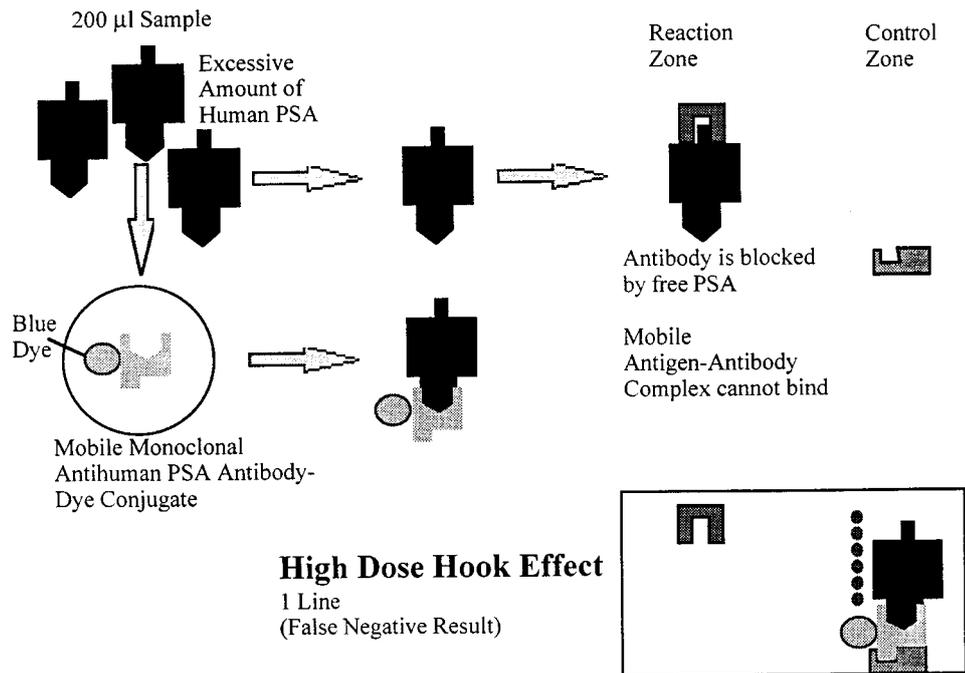


FIG. 2—A complex is formed, concentrating dye particles and also resulting in the formation of a line. The test is considered valid when one line in the control zone is observed. With a positive test result (>4 ng PSA/mL), two lines appear, with a negative result, only the control line is visible.

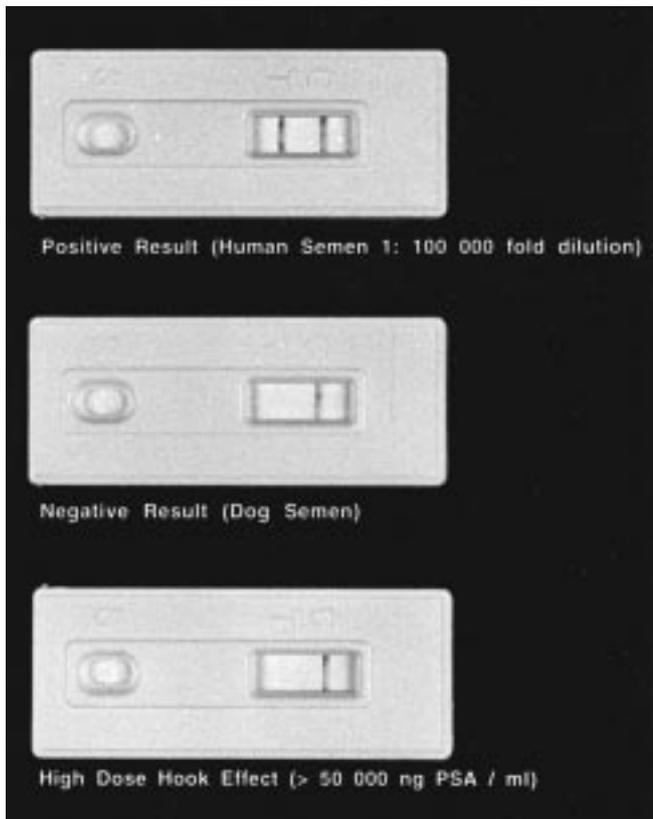


FIG. 3—A membrane test assay for the identification of PSA. Two hundred µL of the extract are placed in the oval opening of the device (S). A positive result is indicated by the formation of a line in the test zone (T) and in the control zone (C). A negative result is indicated by the formation of a line in the control zone only. The high dose hook effect is a false negative result due to excess PSA in the sample and indicated by the formation of a line in the control zone only.

used in this study state, that in serum levels of PSA as low as 4 ng/mL can be detected. Therefore, seminal fluid diluted as much as 1 in 1.3 million may be detectable. In our study all three of the evaluated membrane tests enabled detection of PSA in seminal fluid diluted from 1:50,000–1:1,000 000 offering the same sensitivity of detection as an ELISA based assay.

Samples of six microorganism species, seminal fluid from five mammalian species, various female body fluids, saliva and perspiration from the male donors, and anal swabs all tested negative.

All semen stains on cotton cloth stored at room temperature from 2–30 years tested positive. As expected, some male urine samples tested positive while others tested negative (2,3,5).

Detection of PSA in urine samples can be due to either small amounts of prostatic fluid, to an inflammatory process in prostatitis, or benign prostatic hyperplasia (14). The 50 vaginal swabs from recent casework were first analyzed using a presumptive test for semen (acid phosphatase). Thirty-five tested positive for ACP. All those positive for ACP were also positive for PSA, and seven of the samples negative for ACP were positive for PSA. This confirms observations by Graves et al. describing that PSA antigen was detectable in vaginal fluid for a mean period of 27 h after coitus, as compared with 14 h for prostatic acid phosphatase (3).

Generally, no presence of ACP or low levels of ACP would suggest a lack of semen (due to endogenous levels of ACP sometimes detected in vaginal fluids). Since PSA is not normally present in vaginal fluids (3), detection even at low levels can be indicative of the presence of semen. Therefore the PSA test is a more reliable indicator of the presence of semen.

DNA Compatible Assay

Most forensic laboratories characterize body fluid stains using DNA-based procedures. The procedure, described in the Material and Methods section enables analysis of PSA and DNA and yields results comparable to those that would be obtained if each test were

done individually. DNA was not detected in the supernatant used for the PSA assay. Therefore, no DNA is consumed when assaying for PSA in this manner.

High Dose Hook Effect

A negative result in the PSA test may indicate absence of antigen, antigen below the level of detection, or excess antigen in the sample. As described previously by Hochmeister et al. (15), the latter of these possibilities, resulting in the high dose hook effect, should be considered when performing membrane-based immunoassays. The manufacturers of the tests state that a high dose hook effect has been observed at concentrations of PSA greater than 50,000 ng/mL.

We confirmed this observation and recommend that a dilution of seminal fluid to at least 1:100 should be used to avoid the high dose hook effect. This can be achieved either by extracting a smaller portion of a sample, by increasing the extraction volume, or by diluting the extract prior to assay.

Conclusions

There are many ways to detect PSA in seminal stains. The ELISA is the most sensitive non-membrane based assay and is particularly effective when a large number of samples are processed per day. However, for laboratories with small caseloads, the time and labor to carry out ELISAs can be demanding. All three PSA membrane tests are easier to employ and provide results in a more rapid fashion compared with the traditional PSA detection methods currently used in forensic science. Since PSA membrane tests offer the same sensitivity as ELISA, they present a viable alternative for many forensic laboratories.

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