

# Evaluation of Prostate-Specific Antigen (PSA) Membrane Tests for the Forensic Identification of Semen

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## GOAL

The goal of this study was to evaluate prostate-specific antigen (PSA) rapid membrane tests for the forensic identification of seminal fluid from vasectomized individuals. Rapid membrane test assays offer the same sensitivity as ELISA-based tests and represent a rapid approach for the forensic identification of seminal fluid from vasectomized individuals.

Prostate specific antigen (PSA, also known as p30), a glycoprotein produced by the prostatic gland and secreted into seminal plasma, is now accepted as a marker for detecting semen in criminal cases involving vasectomized or azoospermic males. The reported frequency of azoospermia of 1-9% in seminal stains or swabs examined in sexual assault cases (1) can be expected to rise, since the frequency of contraceptive vasectomy has been estimated to be 750 000 to 1,000 000 per year in the United States (2).

Successful isolation and purification of PSA from human semen (3) has made it possible to develop immunological methods for its detection. Methods for the detection of PSA include Ouchterlony double diffusion, crossover electrophoresis, rocket immunoelectrophoresis, radial immunodiffusion, and ELISA (4). The extremely sensitive ELISA technique can detect PSA in body fluids at concentrations as low as ~4 ng per milliliter. A disadvantage of all techniques is that they are either not sensitive enough or cumbersome and time consuming to perform in forensic laboratories dealing only with a few cases per week.

Various antigen specific membrane tests are currently used in clinical settings to screen a patient's serum for the presence of PSA in levels > 4 ng / ml indicating either benign prostatic hyperplasia or prostatic cancer. All tests are based on the reaction between an antigen and a gold labeled monoclonal antibody. The complex formed migrates through a membrane by capillary forces and reacts with a second membrane fixed monoclonal antibody developing a membrane fixed colored line.

## MATERIAL AND METHODS

Using semen stains stored at room temperature for up to 30 years, postcoital vaginal swabs taken at different time after intercourse, semen free vaginal swabs, and various female and male body fluids including urine, the following PSA specific membrane tests were evaluated: PSA-check-1, VEDA Lab., 61006 Alencon, France; SERATEC® PSA Semiquant, SERATEC, Gesellschaft für Biotechnologie mbH, Göttingen, Germany; One Step ABA card PSA, Abacus Diagnostics, West Hills, CA 91307, USA), and PSA-specific test sticks ("Onestep"; Test Strip, FF Diagnostic, Cologne, Germany). All tests use monoclonal antibodies directed against constant epitopes in free and complexed PSA as well as all its isoforms. Extraction of specimens was performed in 750 µl of HEPES buffered saline for 2 hrs at 4°C (distilled water or other buffers suitable for further DNA extraction may be used as well). This procedure recovers approximately 99% of the extractable PSA on the swab, as demonstrated previously. After a 3 min. centrifugation step 300 µl of the supernatant were removed and 200 µl were used for the PSA test. A positive test result (> 4 ng PSA / ml) is indicated by the formation of a red line in the test and control region of the membrane and the result is read after 10 minutes.

## RESULTS

Using these tests, the reported findings of the detection of PSA in male and female body fluids and secretions could be confirmed. As expected, the membrane tests did not detect PSA in any samples from women. Besides semen from both normal and vasectomized men, positive results were only obtained from post-ejaculate urine and male urine from adult men, when the urine samples were directly added to the membrane tests. However, it is well established that PSA does occur in these fluids. The reliability of these tests in a forensic setting was

confirmed by the analysis of evidentiary material from sexual assault cases known to contain seminal fluid from non-vasectomized or vasectomized individuals. Semen stains stored at room temperature for up to 30 years yielded a positive result. The sensitivity and detection limits of the rapid PSA specific membrane tests are equal to an enzyme-linked immunoabsorbent assay (dilutions of seminal fluid up to 1:1,000,000 are positive).

It is important to notice, that from some semen stains a negative result was obtained, that turned positive when a 1:100 or 1:1,000 fold dilution was retested. It should be kept in mind that a negative membrane test result can be caused by high concentrations of PSA in the extract (causing a so called high hook effect). In these cases a 1:100 or 1:1,000 fold dilution of the remaining 100 µl of the supernatant should be retested.

## **EXAMPLES OF APPLICATIONS**

### **1. Sexual Child Abuse**

Stains on child's clothing are frequently examined for the presence of seminal fluid. When they are found to be negative for sperm cells, a PSA-specific membrane test should be carried out.

We present here a case where no sperm cells were detected, and a PSA-specific membrane test was positive. It was later confirmed that the assailant was a vasectomized individual.

### **2. Rough Time Estimate of Sexual Assault**

It is known, that ACP is detectable in the vaginal tract up to max. 14 hrs., PSA up to max. 14-47 hrs., and sperm cells up to max. several days.

We recently had a case with delayed report 4 days after a sexual assault. Very few sperm cells were found and the PSA test was negative, confirming the patient's claim. A strong positive PSA test would not have been consistent with the reported time frame.

## **CONCLUSIONS**

In conclusion, compared to time consuming ELISA-based measurements of PSA, rapid membrane tests offer the same sensitivity (4 ng PSA / ml) within 10 minutes using 200 µl of supernatant from the DNA extraction procedure. Although test sticks offer the same sensitivity, we found them not useful for casework due to the greater amount of liquid required. Rapid PSA specific membrane tests offer the forensic community a reliable and extremely sensitive tool for the identification of seminal fluid from vasectomized individuals. If the presence of male urine is in question, additional testing using the seminal vesicle specific antigen MHS-5 (SEMA, Humagen Fertility Diagnostics, Inc.) can be useful. These tests can easily be implemented into all forensic casework laboratories (5).

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