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SERATEC® PSA CRIME SCENE BOX

Cat-No. PSM400FE

In-vitro diagnostic test for professional forensic use for the detection of seminal fluid by the semi-quantitative determination of PSA (Prostate-specific antigen)

Intended Use

The SERATEC® PSA Crime Scene Box enables the rapid detection of PSA, a marker of seminal fluid directly at crime scenes. The box includes 20 crime scene Kits containing one PSA test (individually sealed inclusive plastic pipette), one sterile cotton swab and one buffer solution tube (1 mL).

Introduction

PSA (Prostate-specific antigen) is a glycoprotein produced in the prostate and secreted into the seminal fluid. PSA is one of the major proteins in seminal fluid with concentrations of 0.2 to 3.0 mg/ml. Its main function is to liquefy the seminal fluid. This high amount and the fact that PSA is found only at very low concentrations in female vaginal fluid (0.4-0.9 ng/mL and 0.0-1.25 ng/mL, respectively) [2, 3] make PSA an interesting marker in forensic science for the detection of even small amounts of seminal fluid. The advantages of a PSA determination are:

- The detection of PSA is possible in cases where no spermatozoa can be found (for example vasectomized men).
- Very small amounts of PSA can be detected. Studies of MACALUSO et al. (1999) showed that an amount of only 10 µl PSA increased the PSA concentration in vaginal fluid ca. 200 fold.
- PSA shows a good stability. In vaginal smear it is detectable up to 14-47 hours after the intercourse.[5] Also in 30 years old semen stains PSA could be recovered at detectable concentrations.[1]
- PSA is a marker, which is more specific than the acid phosphatase test.

The test will be affected in its evidence by the fact that other body fluids as blood or urine can also contain PSA. Whereas the PSA concentration of male blood serum is normally low (< 4 ng/mL) and is elevated only in the case of prostatic diseases up to 200 ng/mL, the amount of PSA in urine of healthy men showed in some cases values of 800 ng/mL (estimated value).[4] In the case of doubt a differentiation between seminal fluid and urine may be possible by the determination of the PSA concentration. The highest degree of dilution at male urine with a reported positive PSA result is 1:200. On the other hand semen samples generally show positive PSA results even at a dilution factor of 1:200,000 that means at a 1,000 fold higher dilution.[4]
Studies show that low amounts of PSA are already detectable in the urine of eleven years old boys.[4]
For a list of other body fluids that contain PSA, please see reference # 6. Normally the PSA concentrations in the other body fluids are low, so that an interference with the interpretation of the test result is unlikely to expect if working with extracted/diluted materials.

Description of the test

For the detection of semen the test is generally used in a qualitative way. In special cases, however, it might be helpful to estimate the amount of PSA in the sample by correlating the intensity of the test result line with the internal standard, which correlates with an amount of 4 ng/ml PSA.

Principle of the test

The SERATEC® PSA SEMIQUANT test is a chromatographic immunoassay (CIA) for the rapid semi-quantitative determination of PSA in body fluids. It contains two monoclonal murine anti-PSA antibodies as active compounds. One of these antibodies is immobilized at the test region on the membrane. The upstream control region and the region of the internal standard (between control and test region) contain immobilized polyclonal goat anti-mouse antibodies. The amount of antibody at the internal standard is adjusted to a color intensity of the line, which is equal to the color intensity of the test line at a PSA concentration of 4 ng/mL. A glass fiber pad downstream of the membrane is used for sample loading and transmission to a second fiber pad with the dried and gold labeled second monoclonal murine anti-PSA antibody. PSA at the sample will bind to the re-mobilized gold-labeled antibody and form a PSA-gold-labeled-anti-PSA-antibody-complex.
Through the capillary effect of the membrane, the reaction mixture including the complex is carried upwards with the fluid. In any case the colored gold labeled anti-PSA-antibody will bind to the anti-mouse-antibody at the control region and the region of the internal standard thus developing two red lines (one at the control region ant one at the region of the internal standard). These two lines are independent of the existence of PSA in the sample and indicate only the correct execution of the test.
If the sample contains PSA, the PSA-gold-labeled anti-PSA-antibody complex will bind to the immobilized monoclonal antibody of the test result region that recognizes another epitope on the PSA molecule (sandwich complex). The binding is indicated by the formation of an additional line. Thus a PSA **positive** sample will show **three** colored lines in the result window.

Materials

Materials provided:

- 20 crime scene Kits containing one PSA test (individually sealed inclusive plastic pipette), one sterile cotton swab and one buffer solution tube (1 mL)
- one user instruction leaflet

Materials required but not provided: Timer

Storage and Stability

The test is stable up to the expiry date stated on the sealed pouch. The tests can be stored at room temperature or refrigerated (+2 to +30°C). The test must remain in the sealed pouch until use.

Qualitative Characteristics

Sensitivity

The test is capable of detecting PSA in a concentration range of at least 2 ng/mL PSA to 100 µg/ml PSA. Please note that samples containing less than 2 ng PSA/ ml may also produce faint positive results so that 0.5 ng PSA/mL are most of the times still detectable with the test. At ≥500 µg/mL the test result is hampered by an excess amount of PSA resulting in a high dose hook effect.

Reference Preparations

The qualitative characteristics of the test are confirmed in a final QC testing using the following WHO standard: Prostate Specific Antigen (90:10), First International Standard, NIBSC Code 96/670.

Performance Characteristics

The following performance characteristics were observed at a concentration of 2 ng PSA/ml (guaranteed detection limit of the SERATEC PSA SEMIQUANT test)

Diagnostic sensitivity:	100 %	
Diagnostic specificity:		100 %
Positive predictive value:	100 %	
Negative predictive value:	100 %	
Reproducibility:	100 %	

Specificity

The test shows no cross reactivity with other proteins of the seminal fluid. An immunoblot with seminal fluid using the respective PSA antibodies resulted only in one reactive line at the height of PSA. No cross reactivity was observed with the seminal fluid of other mammals (dog, cat, horse, bull, pig)[1] except for the seminal fluid of primates.
No cross reactivity was observed with blood serum. Female blood has a PSA content below the detection limit and showed no reactivity.

Test procedure

Precautions

Seminal fluid and all materials coming in contact with it should be handled and disposed of as if capable of transmitting infection. Avoid contact with skin by wearing gloves and proper laboratory attire. The test and all materials coming in contact with seminal fluid should be autoclaved before their disposal.

- For single IN VITRO DIAGNOSTIC use only.
- Do not use tests after expiration date or if the pouch has been damaged.
- The test consists of potentially infectious materials (e.g. antibodies). These materials do not cause any danger if the device is used according to the instructions.
- Do not open pouches until ready to perform the assay.

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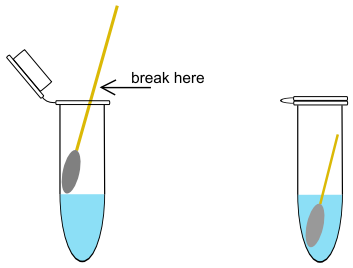
Specimen collection and handling

In General: Seminal fluid should be diluted at least 1:500 prior to use because of its extremely high PSA content For the dilution we strongly recommend to use the provided buffer solution or alternatively a 1 M TRIS solution with a neutral pH value of 8.2.
At the crime scene: Collected semen stains or swabs should be put in the provided buffer tube. Please break the wooden swab so that it fits into the buffer tube for extraction. After shaking for about two minutes, the solution can be used for the test. If the result is negative, the sample should be extracted for a longer time with the remaining buffer to avoid false negative results in case of a low PSA concentration of the sample.

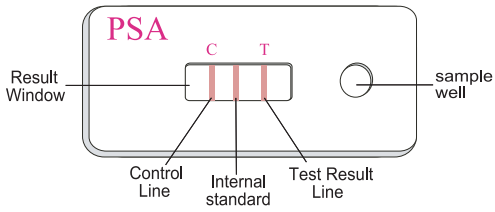


In the laboratory: Semen stains or swabs should be extracted with ca. 250µL buffer about 2 hours by using a shaker. The extractive could be used directly or optionally centrifuged for about one minute at 13,000g. Then 200µL of the PSA containing supernatant is removed and used for the test. If the supernatant is too viscous because of a high PSA concentration, it should be diluted. Particles of tissue do not interfere with the test result.

Note!

- A high viscosity of the sample might interfere with the capillary flow.
- Allow samples to warm up to room temperature before starting the test.
- A pH-value below 2 of the specimen can cause false positive or invalid results.

Start of the assay



- Bring test device to room temperature. Remove from protective pouch and label device for identification purposes.
- Add five drops (about 200 µl) in the sample well. Keep remaining sample in case it might be necessary to test additional dilutions.
- Read result after 10 minutes incubation at room temperature. There should be no remaining fluid in the sample well at this time point. If you want to estimate the amount of PSA by comparison with the internal standard keep strictly to the 10 minutes. Otherwise the intensities of the internal standard and the result may change resulting in incorrect readings.

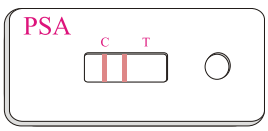
Interpretation of results

PSA **negative** (below detection limit) samples will show **2 lines** in the result well, whereas **PSA positive** samples will show **3 lines**:

Test result line (T): reflects PSA concentration of the sample, visible in PSA-positive samples only
Internal Standard: color intensity correlates with a concentration of approximately 4 ng/mL PSA
Control Line (C): control for possible procedural errors and for the integrity of test components

Negative result (no PSA in the probe or PSA concentration below detection limit)

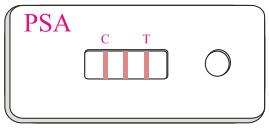
Test result line (**T**) is not detectable. Appearance of internal standard line and control line (**C**) confirm validity of the test. In this case the sample most likely does not contain seminal fluid.



Note:
Make sure that the dilution of the probe leads to a PSA concentration within the detection range. PSA concentrations that are too low (e.g. due to insufficient extraction) or that are too high (e.g. due to insufficient dilution; 500 µg/mL result in a high dose hook effect) interfere with the formation of the test result.

Positive result (PSA detectable)

Test result line (**T**), internal standard line, and control line (**C**) appear.
In this case it is very likely that the sample contains seminal fluid.

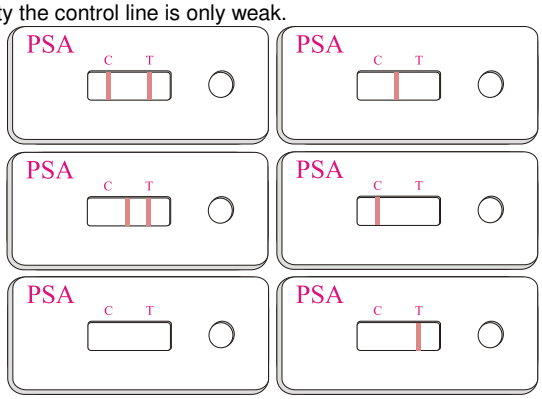


Note:
If there is the risk of mixing up PSA containing body fluids and seminal fluid you might try to get a more accurate result by testing higher dilutions.

Invalid result

Internal standard line and/or control line (**C**) are not detectable. The test is invalid and the assay should be repeated with a new test cassette.

Note:
If the sample contains high amounts of PSA it is possible that the color intensity the control line is only weak.



Suggested reading/References

[1]Hochmeister et al. (1999) Evaluation of Prostate- Specific Antigen (PSA) Membrane Test Assays for the Forensic Identification of Seminal Fluid: J Forensic Sci Vol 44: 1057-1060

[2]Lawson et al. (1998) Objective markers of condom failure. Sex Transm Dis 25: 427-423

[3]Macaluso et al. (1999) Prostate-specific antigen in vaginal fluid as a biologic marker of condome failure. Contraception 59: 195-201

[4]Sato et al. (2002) Use of the „SMITEST" PSA card to identify the presence of prostate –specific antigen in semen and male urine. Forensic Sci Int 127: 71-74

[5]Hochmeister et al. (1997) Evaluation of Prostrate-Specific Antigen (PSA) Membrane Tests for the Forensic Identification of Semen. 8th International Symposium on Human Identification at www.promega.com/geneticidproc/ussymp8proc/33.html

[6]Laux et al., Forensic Detection of Semen III. Detection of PSA Using Membrane Based Tests: Sensitivity Issues with Regards to the presence of PSA in other Body Fluids. http://mafs.net/pdf/forensicdetectionsemen3.pdf

[7]Laux et al., Forensic Detection of Semen II. Comparison of the Abacus Diagnostics OneStep ABA card p30 Test and the SERATEC PSA Semiquant Kit for the Determination of the Presence of Semen in Forensic Cases. http://mafs.net/pdf/laux2.pdf

[8]Gartside et al., Estimation of Prostate-Specific Antigen (PSA) Extraction Efficiency from Forensic Samples Using the SERATEC PSA Semiquant Semiquantitative Membrane Test. Forensic Science Communications 2003 April; 5 (2). http://www.fbi.gov/hq/lab/fsc/backissu/april2003/gartside.htm

[9]SERATEC GmbH: Summary about PSA in body fluids: http://www.seratec.com/docs/user_instructions/psa_in_body_fluids

Symbols

Expiry date

Storage temperature

LOT Lot number

Rev: March 2011