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 SEMIQUANT test serves the rapid detection of PSA in seminal fluid. The interpretation occurs visual by the recognition of a test line in the case of a PSA positive sample. If required the intensity of the test line can be correlated to the intensity of an internal standard which is adjusted to resemble the color intensity of the test result line at 4 ng PSA / 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)²³ 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 seminal fluid 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.² 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,¹ the 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,¹ the PSA concentration of female blood serum is normally low (< 0.0 ng/mL).²³ Female blood serum has a PSA content below the detection limit and showed no reactivity.

### 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 reimmobilized 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 and 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. The line in the middle (internal standard) correlates with an amount of 4 ng/ml PSA. In some cases it might be helpful to estimate the amount of PSA in the sample by comparison of the test result line with the internal standard line.

### Storage and Stability
The tests and the buffer are stable up to the expiry date stated on the sealed pouch resp. buffer tube. Tests and buffer 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

#### 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 blood serum. Female blood has a PSA content below the detection limit and showed no reactivity.

### Materials

#### Materials provided:
- 40 individually sealed PSA test, inclusive plastic pipette
- 50 ml buffer solution
- user instruction leaflet

#### Materials required but not provided:
- Timer

### Miniaturized PSA Test PSA SEMIQUANT
Cat-No. PSM406F

SERATEC® PSA SEMIQUANT USER INSTRUCTION
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.
- Do not freeze the tests.

Specimen collection and handling
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. Semen stains or swabs should be extracted with an appropriate amount of buffer about 2 hours by using a shaker. Very old or small stains may be extracted longer, fresh and strong stains shorter. After centrifugation for about one minute at 13,000g, 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 three drops (about 120 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

8 Gartsie et al., Estimation of Prostate-Specific Antigen (PSA) Extraction Efficiency from Forensic Samples Using the SERATEC® PSA Semiquantitative Membrane Test. Forensic Science Communications 2003 April; 5 (2). http://www.fbi.gov/hq/lab/fsclab/unpub/2003/gartsie.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 number

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