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SERATEC® PAM Test for the simultaneous detection of PSA and Amylase

REF: PAM, PAM/8, PAM/40

Application

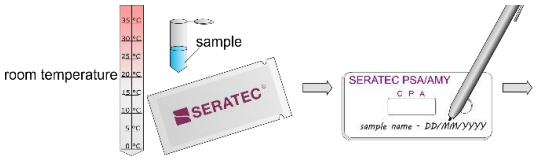
SERATEC® PAM Test is a chromatographic immunoassay for rapid detection of prostate-specific antigen (PSA) and alpha amylase to identify seminal fluid and saliva in forensic samples. The product contains two monoclonal anti-human PSA and two monoclonal anti alpha-amylase antibodies as active components.

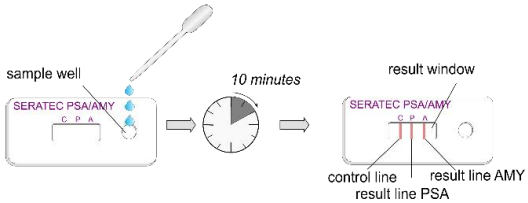
Materials

- 8 or 40 (PAM/8, PAM/40) individually packaged tests in cassette format with one plastic pipette each
- 15 or 50 ml (PAM/8, PAM/40) dilution buffer
- Instructions for use

Additionally required: stopwatch or timer

Test Performance



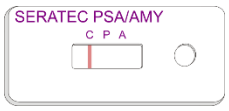


1. Bring all test components to room temperature before performing the test. Low temperatures can lead to a decrease in sensitivity.
2. Remove the test cassette from the pouch and label it for identification.
3. Add 3 drops of the sample (approx. 120 µl) to the sample well with the enclosed plastic pipette and start the time measurement.
4. Read the test result after 10 minutes at room temperature. The liquid in the sample well should have been completely absorbed.
5. Keep the remaining sample material to perform further testing if necessary.

Interpretation of results

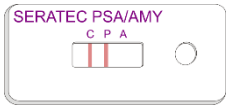
After 10 minutes, up to three lines may be visible in the result window:
PSA result line (P): only visible when the sample is PSA-positive; the colour intensity of the line may vary and depends on the PSA concentration of the sample.
AMY result line (A): only visible when the sample is alpha-Amylase-positive; the colour intensity of the line may vary and depends on the alpha-amylase concentration of the sample.
Control line (C): Control for potential application errors and for the integrity of the test components. This line is always visible after successful performance of the test.

Negative result (PSA and AMY are not detectable; no PSA and AMY in the sample or concentration below the limit of detection):



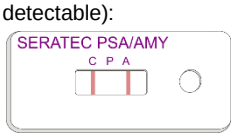
A single line is visible in the result window. The test result lines (P and A) are not present. The appearance of the control line (C) confirms that the test has been performed correctly.

Positive result for PSA (only PSA detectable):



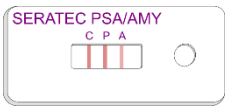
Two lines are visible in the result window: the PSA result line (P) and the control line (C). Any visible T-line, regardless of intensity, indicates a positive result. No line appears for AMY (A).

Positive result for AMY (only AMY detectable):



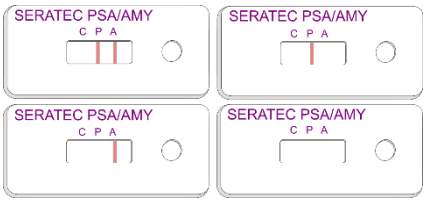
Two lines are visible in the result window: the AMY result line (A) and the control line (C). Any visible T-line, regardless of intensity, should be considered a positive result. No line appears for PSA (P).

Positive results for PSA and AMY (PSA and AMY detectable):



Three lines are visible in the result window: the PSA result line (P), the α-Amylase result line (A), and the control line (C). Any visible T-line, regardless of intensity, should be considered a positive result.

Invalid result (no usable result):



No control line (C) is visible, indicating an invalid test. The test should be repeated using a new cassette.

Sample preparation

In order to obtain optimal test results, follow these instructions:

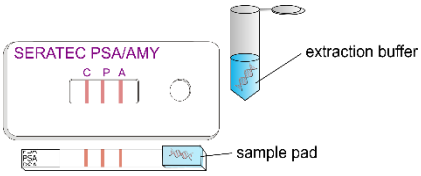
- It is not recommended to use unknown samples undiluted. Liquid samples should be diluted at least 1:500 prior to testing. [1]
- Viscous samples should be diluted until the sample flows smoothly on the test membrane.
- Use the buffer solution included in the scope of supply, as it has been developed specifically for the Test. Other buffer solutions or the use of water may result in reduced sensitivity or fluctuating line intensities.
- Do not use liquids with a pH below 3 or above 12, as this may cause incorrect or invalid results.
- Tissue particles do not affect the test result.
- Cotton swabs, cloth or condom pieces should be extracted in a sufficient amount of buffer. The cut piece should be between 0.25 and 1 cm^2 in size and should be extracted in approx. 0.5 – 1 ml buffer.
- An extraction time of approx. 10 minutes is recommended. You should however follow the rule that the older or smaller the stain, the longer the recommended extraction time.[2]
- Extracted samples are stable at room temperature for about 2 days. Samples kept for longer periods should be stored dry and cold (2 – 8 °C). Liquid samples may be frozen.

Dilution buffer

The supplied dilution buffer contains the following constituents (in 1 l distilled H_2O):
8,0 g NaCl; 0,2 g KCl; 1,44 g $Na_2HPO_4 \cdot 2H_2O$; 0,24 g KH_2PO_4; 0,1 ml 10 wt% NaN_3; pH 7,4.

DNA profiling

The extracted samples can be stored for further analyses (e.g. DNA profiling; see Sample preparation).
The extracted sample is compatible with DNA analyses. It is also possible to extract DNA from the sample pad for further analysis.[3,4]



Safety information

Forensic samples are potentially infectious material which should be examined with the appropriate care and only when suitable protective measures (e.g. gloves, laboratory clothing) are applied. Materials used to perform the test should be autoclaved before disposal, as they contain potentially infectious material. Observe the following instructions:

- Do not use the product if damaged.
- Only remove the test cassette from the pouch immediately before use.
- Do not use the product after the expiration date.
- The materials used in the test (e.g. antibodies) are potentially infectious materials. When used and disposed of properly, however, there is no danger to the user or others.
- Do not freeze the test cassette.

Background PSA

Prostate-specific antigen (PSA) is a glycoprotein produced in the prostate. It is secreted into the seminal fluid for liquefaction and reaches concentrations of 0.2 to 3.0 mg/ml. These high levels as well as the fact that PSA only occurs in very low concentrations (0.0 – 1.25 ng/ml[5,6]) in female vaginal secretions make **PSA a suitable marker for the detection**

of small quantities of seminal fluid. The benefits in forensic application compared to other detection methods are:

- Easy handling without additional equipment – directly at the crime scene or in the laboratory.
- A quick and reliable result after 10 minutes.
- Detection of PSA is also possible in cases where no sperm cells can be found (e.g. after a vasectomy).[7]
- High stability of PSA – positive detection could be obtained with 30-year-old samples.[7]
- Detection of PSA in vaginal swabs up to 27 hours after sexual intercourse.[5,7]
- Higher specificity of PSA for the detection of seminal fluid compared to "acid phosphatase tests".[6,7]
- In simulated samples of vomit, PSA was detectable for up to 4 hours.[8]
- Greater reliability in the detection of PSA in vaginal swabs compared to semenogelin.[9]

Note: Besides seminal fluid, PSA occurs in other body fluids and secretions/excretions, e.g. blood, urine, stool.[10,11] The recommended sample dilution (see sample preparation) reduces the probability that samples not containing any seminal fluid show a positive test result. More information on PSA in body fluids as well as recommendations have been gathered by the manufacturer in a freely available document and can be found in the references. [1,2,12]

Background Amylase

The enzyme α-amylase is used in the body to break down polysaccharides and occurs in various organs and body fluids. Its concentration in saliva and pancreatic fluid is particularly high. The α-amylase found in saliva (also called ptyalin) initially breaks down insoluble starch into soluble forms (amylodextrin, erythrodextrin and achrodextrin), then breaking those down further into maltose.

There are several methods for the detection of saliva in forensic sample material by means of detecting α-amylase. Saliva tests that do not directly detect human α-amylase, but its activity (e.g. Phadebas), may indicate positive results regardless of the origin of the amylase (human, animal, plant). The SERATEC® PAM Test features **high sensitivity and specificity** and the **detection of human α-amylase as a marker of saliva** offers the following benefits in forensic applications:

- Easy handling without additional equipment – directly at the crime scene or in the laboratory.
- A quick and reliable result after 10 minutes.
- High specificity through direct detection of human α-amylase (see Specificity).

Note: Since α-amylase can also occur in other body fluids and secretions/ excretions, e.g. blood, urine, stool, seminal fluid, vaginal fluid, these samples may show a positive test result. The recommended sample dilution (see Sample preparation) reduces the probability that samples not containing any saliva show a positive test result. Please note that stool contains saliva amylases because of natural swallowing of saliva. For this reason, stool samples may show a positive test result. Breast milk likewise contains α-amylase and can cause a low positive test result in large concentrations. Overall, breast milk reacts about 20 times weaker than saliva. Pure urine samples may also cause a positive result. In this case, however, a dilution by 1:200 already leads to negative results. More information and recommendations on the use of the SERATEC® PAM Test in forensic biology have been gathered by the manufacturer in a freely available document.[14]

Sensitivity

Sensitivity for PSA: Quantities of min. 1 ng/ml human PSA are detectable. The **high dose hook effect** will not impact a positive test result. Seminal fluid diluted in the range between 1:1 and $1:10^6$ is successfully detected in the recommended dilution buffer.

Sensitivity for AMY: Quantities of min. 50 mIU/ml human α-amylase detectable. The **high dose hook effect** will not impact a positive test result. Human saliva diluted in the range between 1:1 and $1:10^3$ is successfully detected in the recommended dilution buffer.

Specificity

For PSA: The test does not show any cross-reactivity with other proteins in seminal fluid. No cross-reactivity was observed with seminal fluid from other mammals (dog, cat, horse, bull, pig, ram and others). [7,13] A possible exception is seminal fluid from higher primates, but no data on cross-reactivity are available.

For Amylase: The test does not show any cross-reactivity with other proteins in saliva. No cross-reactivity has been observed with the saliva of various animal species (dog, cat, rabbit, horse, cow, pig, mouse, goat, sheep, hamster and others). A probable exception is saliva from higher primates, but no data on cross-reactivity is available. *

Storage and shelf life

- Store test cassettes and buffer solution at +2 to +30 °C (38 to 86 °F).
- Keep test cassettes in the pouch until use.
- Do not use after the specified expiration date.

Quality features

Our products are manufactured according to the quality standards of European standard ISO 9001. The performance characteristics are confirmed during final quality control in application of the following standards: *PSA NIBSC Code 96/668 and for AMY 17/102. α-Amylase from human saliva* (Lee Biosolutions, 120-10 *or* Sigma Aldrich, A1031).

Please contact us if you have any questions or require more information.

Literature

[1] D.L. Laux, S.E. Custis, 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, in: 2004.

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[4] H. Holtkötter, C.R. Dias Filho, K. Schwender, C. Stadler, M. Vennemann, A.C. Pacheco, G. Roca, Forensic differentiation between peripheral and menstrual blood in cases of alleged sexual assault—validating an immunochromatographic multiplex assay for simultaneous detection of human hemoglobin and D-dimer, Int. J. Legal Med. 132 (2018) 683–690. doi:10.1007/s00414-017-1719-y.

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[10] S. Bolduc, L. Lacombe, A. Naud, M. Grégoire, Y. Fradet, R.R. Tremblay, Urinary PSA: a potential useful marker when serum PSA is between 2.5 ng/mL and 10 ng/mL, Can. Urol. Assoc. J. J. Assoc. Urol. Can. 1 (2007) 377–381.

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

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[14] SERATEC GmbH, Evaluation of the SERATEC AmylaseTest

Symbols


Expiry date


Storage temperature

Lot number