SERATEC®
Gesellschaft für Biotechnologie mbH

SERATEC® AmylaseTest: An Overview for Users

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This overview should help product users to interpret test results easier. It contains various data from several SERATEC studies conducted as part of the product development and validation process. Futhermore, there are recommendations given on sample preparation and result analysis.

1) Background

Amylase is found in saliva and breaks starch down into maltose and dextrin. This form of amylase is also called "ptyalin". It breaks large, insoluble starch molecules into soluble starches (amylodextrin, erythrodextrin, achrodextrin), producing successively smaller starches and ultimately maltose. Ptyalin acts on linear α(1,4) glycosidic linkages, but compound hydrolysis requires an enzyme that acts on branched products.

2) Short Description of the SERATEC® AmylaseTest

General

The SERATEC® AmylaseTest is a chromatographic immunoassay (CIA) for the rapid determination of α-Amylase in forensic samples. It contains two monoclonal murine anti- α-Amylase antibodies as active compounds. One of these antibodies is immobilized at the test region on the membrane. The upstream control region contains immobilized polyclonal goat anti-mouse antibodies. A glass fibre pad downstream of the membrane is used for sample loading and transmission to a second fibre pad with the dried and gold labelled second monoclonal murine anti- α-Amylase antibody and a gold labelled mouse antibody for the control line. α-Amylase in the sample will bind to the gold-labelled antibody and form an antigen-gold-labelled-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 labelled mouse-antibody binds to the anti-mouse-antibody in the control region, resulting in the appearance of a red line. This red line comes up regardless of the presence of α-Amylase in the sample and solely indicates the correct execution of the test.

If the sample contains α-Amylase, the α-amylase-gold-labelled-anti- α-amylase-antibody complex binds to the immobilized monoclonal antibody located in the result region by recognizing another epitope on the α-amylase molecule and forming a so-called *sandwich complex*. This becomes visible as a second red line.

SERATEC® AmylaseTest at a glance:

Intended Use:	Detection of human saliva by determining α-Amylase
Principle:	Chromatographic Sandwich Immunoassay
Range:	Lower Detection Limit: 50 mIU/mL ~ 0.2 µL saliva/Test
Time:	10 minutes after addition of the sample the test result is interpreted visually
Laboratory Kit:	Box with 40 individually wrapped cards including pipettes and desiccant, one vial 50 ml of dilution buffer, one user instruction leaflet
Crime Scene Kit	20 Kits of one card (pouched with pipette and desiccant), one DNA free pushoff swab for sample collection, one vial with dilution buffer (1 mL) and one user instruction leaflet

Recommendations for the Test Procedure

Specimen collection

The extraction of old stains may be difficult. If necessary, the extraction can be done in the laboratory with the help of a shaker. As solvent we strongly recommend the use of the provided extraction buffer. Also, other buffers with a neutral pH range (e.g. Phosphate, HEPES or TRIS buffered saline) may be used. If necessary, the extraction buffer can be prepared as follows: Solve in 200 mL di. water: 8.0g NaCl; 0.2g KCl; 1.44g $Na_2HPO_4 \bullet 2H_2O$; 0.24g KH_2PO_4; 0.1mL 10wt.% $NaN_3 \rightarrow$ fill with distilled water to 1000 mL, adjust pH to 7.4 with HCl and/or NaOH.

Test Precedure

Allow all test components to warm up to room temperature before starting the test.
Remove the cassette out of the foil pouch and tag the cassette with a marker, if necessary.
Add three drops (about 120 µl) in the sample well. Keep remaining sample if possible, in case it might be necessary to test additional dilutions.
Read result after 10 minutes incubation time at room temperature. There should be no remaining fluid in the sample well at this time point.

Dependence on the pH

Studies in our laboratory showed that the result of the SERATEC® AmylaseTest is influenced by the pH of the sample material. Low pH values (pH < 3) caused by **organic acids** (citric acid, acetic acid, oxalic acid etc.) might lead to invalid or false positive results. Frequently, the line is spotted and is not formed uniformly across the whole width of the membrane surface. Interestingly, this phenomenon is **only** observed **at low pH in the presence of organic acids**. If the pH of other buffer solutions (see table below) is adjusted with HCl, no false positive results are observed (up to pH 3). Between pH 5 to pH 10 no invalid or false positive results occur in any case. In this range the test sensitivity remains constant.
In any case we recommend preparing the sample material in a way, that the pH is neutral or close to neutral. If possible, please use the buffer included in the kit. When using other buffer standards, it is not recommended to use distilled water for the extractions in order to avoid changes in the pH. In some cases it might be necessary to check the pH of the liquid with a small piece of pH indicator paper.

3) Test procedure

Specimen collection and handling

Liquid samples should be diluted at least 1:10 prior to use because of the high viscosity of saliva. For the dilution we recommend to use the provided PBS based buffer (good results were additionally achieved with TRIS buffered saline and HEPES buffer). Stains or swabs can be extracted with buffer by incubating them on a shaker. Particles of tissue do not interfere with the test result.

Test Sensitivity

The sensitivity of the saliva test was determined by the use of saliva samples. The table below shows the average values of 10 male and 10 female test persons. The tests were repeated five times per person.

Test sensitivity in different extraction buffers:

Parameter	conc.	result (TRIS)	result (PBS)	result (Hepes)
saliva (♂)	1/10	positive	positive	positive
saliva (♂)	1/100	positive	positive	positive
saliva (♂)	1/1000	positive	positive	positive
saliva (♂)	1/5000	negative	negative	negative
saliva (♀)	1/10	positive	positive	positive
saliva (♀)	1/100	positive	positive	positive
saliva (♀)	1/1000	positive	positive	positive
saliva (♀)	1/5000	negative	negative	negative

4) Cross reactivity screening with different body fluids

Although found in many tissues, amylase is most prominent in pancreatic juice and saliva, each of which having its own isoform of human α-amylase. The average value for adults in serum are 100mIU/mL and for urine 460 mIU/mL.

Test results with blood and serum:

Parameter	conc.	result (TRIS)	result (PBS)	result (Hepes)	result (di. H_2O)
blood	1/10	negative	negative	negative	negative
blood	1/100	negative	negative	negative	negative
blood	1/1000	negative	negative	negative	negative
serum	1/10	negative	negative	negative	negative
serum	1/100	negative	negative	negative	negative
serum	1/1000	negative	negative	negative	negative

Test results with urine:

buffer	conc.	result (♂)	result (♀)
/	neat	positive	positive
TRIS	1/10	negative	negative
PBS	1/10	negative	negative
Hepes	1/10	positive	positive
Hepes	1/15	negative	negative
di. water	1/10	negative	negative

Test results with seminal fluid:

buffer	conc.	result
TRIS1	1/10	negative
TRIS2	1/10	negative
PBS	1/10	negative
Hepes	1/10	negative
di. water	1/10	negative

5) Cross reactivity screening with different species

Fortunately we could not observe a cross reactivity with the saliva of domestic animals except for guinea pigs. The saliva samples were collected by a veterinarian and then directly tested after extraction over 2 hours in the respective buffer solution.
It is verisimilar that saliva of upper primates reacts positive with the test. These additional specificity tests will be subject of an extra evaluation study.

Test results of the species specifity testing:

species	Sample	conc.	result (TRIS)	result (PBS)	result (Hepes)
dog	cotton swab	swab/1mL	negative	negative	negative
cat	cotton swab	swab/1mL	negative	negative	negative
rabbit	cotton swab	swab/1mL	negative	negative	negative
horse	cotton swab	swab/1mL	negative	negative	negative
mouse	cotton swab	swab/1mL	negative	negative	negative
domestic pig	cotton swab	swab/1mL	negative	negative	negative
goat	cotton swab	swab/1mL	negative	negative	negative
cow	pure saliva	1/10	negative	negative	negative
cow	cotton swab	swab/1mL	negative	negative	negative
guinea pig	cotton swab	swab/1mL	positive	positive	positive
hamster	cotton swab	swab/1mL	negative	negative	negative
sheep	cotton swab	swab/1mL	negative	negative	negative

6) Summary

The SERATEC® AmylaseTest is a suitable device for the detection of human α-Amylase. Best results were obtained with PBS buffer, which is part of the test kit.
Cross reactivity tests with further biological materials like breast milk, Amniotic fluid, feces, perspiration and vaginal fluid will be done in the near future.

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