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Gesellschaft für Biotechnologie mbH

SERATEC® AmylaseTest

An Overview for Users

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

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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. Description of the SERATEC AmylaseTest

General

The SERATEC® Saliva test is a lateral flow immunoassay for the rapid determination of α-Amylase in forensic samples. It contains two monoclonal 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 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 anti-α-Amylase antibody and a gold labelled polyclonal antibody for the control line. α-Amylase in the sample will bind to the gold-labelled anti-α-Amylase 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 coloured gold labelled polyclonal-antibody binds to the polyclonal-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 which corresponds to ~ 0.1 µL saliva/Test
Time:	10 minutes after addition of the sample the test result is interpreted visually
Unit:	Box with 40 individually wrapped test devices including pipettes and 50 ml buffer solution

2.1 Methods and Materials

The tests have been performed using SERATEC AmylaseTest. For extractions or dilutions of samples the provided PBS buffer was used unless another buffer was mentioned.
The composition for 1000mL PBS buffer is as follows:
Solve in 200 mL di. water: 8.0g NaCl; 0.2g KCl; 1.44g $Na_2HPO_4 \cdot 2H_2O$; 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.

Other buffers:
Tris buffer composition: 1M TRIS at pH 8.2 with 0.01wt%. NaN_3
Hepes buffer composition: 0.01M Hepes with 0.01wt%. NaN_3

The sample Material for blood and seminal fluid was purchased by SERATEC.
Saliva, breast milk, sweat, nasal secretion and feces have been donated by volunteers.
The animal saliva samples were donated by a veterinarian.

All experiments were carried out in the Laboratory of SERATEC GmbH except the vaginal fluid and the DNA extraction experiments.

3. Recommendations for the Test Procedure

3.1 Specimen collection

Liquid samples should be diluted at least 1:10 prior to use because of the high viscosity of saliva. For the dilution we recommend using 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.
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.

3.2 Test Procedere

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.

3.3 Influence of the pH value

If the test is carried out correctly using the provided buffer solution, it is very unlikely that the test result will be affected by acidic or alkaline samples.
The validation Data showed that the result of the AmylaseTest is influenced only by very low pH values (pH < 3) as well as very high pH values (pH > 12). Liquids with these pH values may lead to invalid or false positive results. Such pH levels can occur by using distilled water instead of the provided buffer solution for extraction. **Organic acids** (citric acid, acetic acid, oxalic acid etc.) could be a reason for low pH values in samples, detergents for high pH values.
For this reason, we recommend using the provided buffer solution for extraction of swabs or cuttings and for dilution of liquid samples. It is not recommended to use distilled water for the extractions in order to avoid changes in the pH value.

4. Sensitivity and standard material

The AmylaseTest sensitivity is 50 mIU/mL of human salivary α-Amylase. For the Quality Control the following standard is used: α-Amylase from human saliva, supplied by Lee Biosolution, Catalogue Number 120-10.

The sensitivity of the AmylaseTest was additionally determined using 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.

Parameter	conc.	PBS (provided buffer)	TRIS	Hepes
saliva (♂)	1/10	positive	positive	positive
saliva (♂)	1/100	positive	positive	positive
saliva (♂)	1/1000	positive	positive	positive
saliva (♂)	1/2000	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 (♀)	1/2000	negative	negative	negative
saliva (♀)	1/5000	negative	negative	negative

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5. Specificity 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.

Specificity screening with Blood and serum samples:

Test Procedure: blood and serum samples were collected and then diluted with the provided buffer solution.

Parameter	conc.	PBS provided buffer	TRIS	Hepes	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

Specificity screening with Urine samples:

Test Procedure: urine samples were collected and then diluted with different buffer solutions.
In addition, cotton swabs were wetted with urine, dried and then extracted into 0.5 mL buffer solution.

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

buffer	conc.	result (♂)	result (♀)
PBS provided buffer	0.5 mL extract	negative	negative

Specificity screening with Seminal fluid samples:

buffer	conc.	result
PBS provided buffer	1/10	negative
TRIS	1/10	negative
Hepes	1/10	negative
di. water	1/10	negative

Specificity screening with Sweat samples:

Test procedure: 3 Different male and female sweat samples were collected with a swab. The swabs were then extracted in 0.5 mL of the provided buffer solution.

buffer	conc.	result (♂)	result (♀)
PBS provided buffer	0.5 mL extract	negative	negative

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Specificity screening with Nasal secretion samples:

Test procedure: Nasal secretion samples from two males and two females were collected with a swab. The swab was then extracted in 0.5 mL of the provided buffer solution.

buffer	conc.	result (♂)	result (♀)
PBS provided buffer	0.5 mL extract	negative	negative

Specificity screening with Vaginal fluid samples:

In a study of Conte et al [1] a total of 50 vaginal swabs from unique donors were collected. The study was the first large-scale vaginal fluid study focused on the results of amylase and PSA immunochromatographic testing results. The Amylase Test reacted negative with 92% and positive with 8% of the samples, indicating a significant result ($p > 0.05$). The four positive test results for amylase were quantified with markers that target total human DNA and total human male DNA. The quantity of total human DNA varied between samples; however, no male DNA was detected.

Specificity screening with Breast Milk samples:

Breast milk contains low-levels of salivary α-amylase that helps breastfeeding babies to digest carbohydrates. Breast milk was directly diluted using the provided buffer solution.

conc.	result (♂)
1/10	positive
1/100	positive
1/200	negative
1/300	negative

The table shows that breast milk only leads to positive results to a dilution of 1/100. Forensic samples usually have a higher dilution, so it is very likely that a negative result can be assumed.
Example: Extraction of a sample in 200µL or more.

Specificity of Feces samples:

Fecal stains may contain levels of **α-Amylase** as high as those found in saliva. For this reason, positive results with samples obviously contaminated with feces should be interpreted carefully. The positive result may be caused by feces or by a mixture of feces and saliva. The presence of potential fecal material in samples should be recorded and included in the result interpretation.

6. Specificity 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.

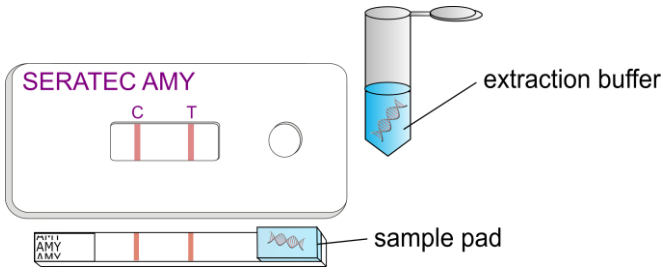
Test results of the species specificity testing:

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

7. DNA Profiling using the Buffer solution and the test strip

The extracted sample (buffer solution) is compatible with DNA analysis. [2] It is also possible to extract DNA from the sample pad for further analysis. [3]



8. References

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