Evaluation results of the SERATEC Amylase Test

An Overview For Users Of the SERATEC® Amylase Tests

Table of Contents

We would like to give an overview about the properties of the SERATEC® Saliva test with a short summary of data performed in several studies at SERATEC. Further we would like to give recommendations concerning the sample preparation and the interpretation of results.

1) Background

Amylase is found in saliva and breaks starch down into maltose and dextrin. This form of amylase is also called "ptyalin". It will break 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 Saliva Test

The SERATEC® Saliva test 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 will bind to the anti-mouse-antibody at the control region thus developing one red line. This red line is independent of the existence of α-Amylase in the sample and indicates only the correct execution of the test.
If the sample contains α-Amylase, the α-amylase-gold-labelled-anti- α-amylase-antibody complex will bind to the immobilized monoclonal antibody of the test result region that recognizes another epitope on the α-amylase molecule (sandwich complex).

Brief Description of the SERATEC® Amylase Saliva Test

Intended Use:	Detection of Saliva by the Determination of α-Amylase
Principle:	Chromatographic Sandwich Immunoassay
Range:	Lower Detection Limit: ~ 0.2 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

- **General recommendations for the test procedure**

The test procedure is started by the addition of 120 to 200 µl of liquid into the round sample well. After an incubation time of 10 minutes at room temperature during which the red lines appear, the test result is interpreted visually. **We generally recommend a 1M TRIS solution with a pH of 8.2 as buffer solution.**

Generally the sample material will be generated by the extraction of swabs or dried stains. Because the expected amount of Saliva, the place from which the sample has been taken, the age of the material etc. will vary considerably; it is difficult to give general recommendations concerning the preparation of sample material.

Dependence on the pH
Studies in our laboratory showed, that the test result of the Saliva test is influenced by the pH of the sample material. Low pH values (pH < 3) that are caused by **organic acids** (citric acid, acetic acid, oxalic acid etc.) might lead to invalid or false positive test results. Frequently the line is spotted and is not formed uniformly across the whole width of the test membrane. 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 sensitivity of the test 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 we strongly recommend using the provided buffer. If this is not possible, other buffer solutions are favored instead of distilled water for the extraction to avoid changes in the pH due to the sample material. 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**

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 TRIS based buffer (good results were additionally achieved with Phosphate buffered saline as dilution 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 buffer systems:

Parameter	conc.	result (SVP)	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 28-100mIU/mL and for urine 460 mIU/mL.

Test results with blood and serum:

Parameter	conc.	result (SVP)	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 for 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 for 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 from upper primates reacts positive with the test. SERATEC plans to evaluate this in an extra study.

Test results of the species specifity testing:

species	Sample	conc.	result (SVP)	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 Saliva test is d suitable device for the detection of human α-Amylase. Best results were obtained with a 1 M pH 8.2 TRIS buffer, which will be a part of the test kit.
Further biological materials like breast milk, Amniotic fluid, feces, perspiration and vaginal fluid are still under investigation.

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