

## Developmental Validation of SERATEC<sup>®</sup> AmylasePaper for the Preliminary Detection of Saliva on Samples of Forensic Evidence

Dr. Christian Stadler, Dr. Gabriela Roca, and Michelle Chan

SERATEC<sup>®</sup> Gesellschaft für Biotechnologie mbH  
Ernst-Ruhstrat-Straße 5, 37079 Göttingen, Germany

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## Introduction

Saliva is one of the common body fluid found in various forensic samples, and its presence can be crucial in criminal investigations. The detection of saliva is one of the primary objective for forensic serologists as it may demonstrate direct contact between the accused and complainant. Testing for saliva is usually required when investigating alleged oral assaults and may include swabs and clothing. The SERATEC<sup>®</sup> AmylasePaper is a mapping tool for the fast preliminary detection of saliva stains on samples of forensic evidence such as t-shirts and underwear.

## SERATEC<sup>®</sup> Amylase Paper

The enzyme  $\alpha$ -amylase is found at very high levels in human saliva. Its ability to digest starch is used as an indicator for the presence of saliva. The SERATEC<sup>®</sup> AmylasePaper is a filter paper impregnated with starch. It detects amylase via the starch-iodine reaction. In the presence of amylase, starch is digested, so that a starch-iodine complex cannot be formed. Thus, areas of the Amylase Paper that have been in contact with amylase are differently colored than those without amylase.<sup>1,2</sup>

## Aim

To make interpretation of results obtained from SERATEC<sup>®</sup> AmylasePaper easier, a summary of data is provided, followed by recommendations regarding sample preparation. Generally, the test objects are in the form of textile stains where the age of the material will vary, affecting traces of samples left behind. Additionally, a user guide with records of visual observation of possible positive and negative result patterns is presented.

## Test Procedure

### General

The test procedure is started by placing the test object on the moistened AmylasePaper laid on a flat surface, followed by pressing down for 10-15 seconds. After an incubation of 10 minutes following the removal of test object, the iodine Working Solution is poured over the tested area on the AmylasePaper and the result is interpreted visually.

### Interpretation of Results

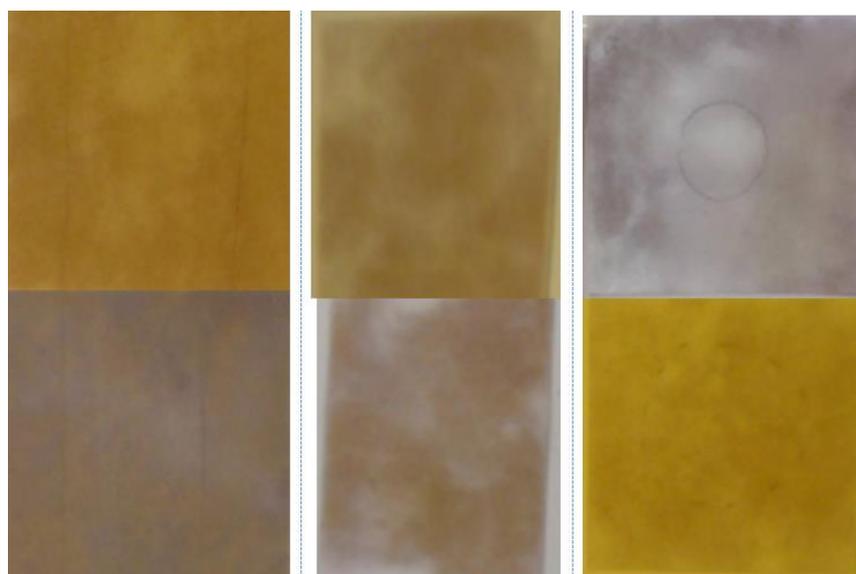
Negative samples show no spot(s) or area of vibrant yellow, whereas positive samples show a vibrant yellow area or spot(s) on the AmylasePaper, usually surrounded by a brownish-blue background. The colour may fade in intensity 5 minutes after initial contact but it remains visually distinct.

A negative result indicates that no amylase is present in the sample or amylase concentration is below detection limit. A vibrant yellow area usually surrounded by a brownish-blue background indicates a presumptive positive result for saliva as Amylase has been detected. A positive result may show spots of different shapes and shades. Air bubbles underneath the AmylasePaper may result in brighter areas in contrast to the surrounding.

The visual guides below illustrate the variety of positive and negative results that may be observed, depending on the size of the object, its type and the Amylase concentration.



*Image 1: Examples of positive results*



*Image 2: Examples of negative results*

## Recommendations

### Preparation of Iodine Working Solution

As the iodine working solution is important in visualising the final results, it is necessary to use an adequate amount for optimal outcome. Studies in our laboratory have shown that a surface area to volume ratio of 10cm<sup>2</sup>:1ml of iodine working solution is optimum for a clear display of positive results. An A4 sheet of paper has a surface area of 619.5cm<sup>2</sup> thus an optimum volume of working solution corresponds to approximately 57.4ml.

The use of inadequate iodine working solution (i.e. less than the advised) would result in faint spots (if any) which could be easily mistaken for air bubbles given the lightness of both areas.

### Transferring of Sample from Test Object to AmylasePaper

Proper transfer of sample from test object to AmylasePaper is crucial in ensuring optimal and reproducible results. It has been observed that, for textile test objects, it is most optimal when

transferring is done by pressing the area of interest directly onto AmylasePaper on a flat, even surface, preventing the rapid diffusion of sample due to gravitational force as opposed to when the AmylasePaper is re-inverted after pressing it on the tested area, as seen in image 3.

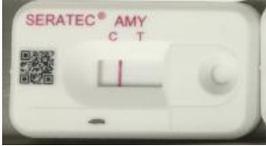
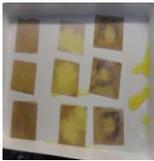


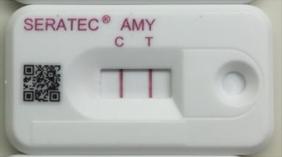
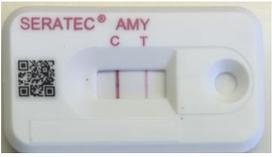
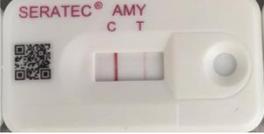
*Image 3: Inconclusive result (left) obtained from inverting and pressing of moist AmylasePaper on textile stained with saliva, as opposed to a positive result (right) obtained from directly pressing of the textile stain onto AmylasePaper.*

### Extra User Guide

Other textile stains have been mapped with the SERATEC<sup>®</sup> AmylasePaper for the presence of saliva. The following tables briefly summarizes the available data.

#### Patterns of Positive Results

Sample Type/ AmylaseTest (protein control)	Sample Carrier	Repetitions	Average Results	Pictures
Negative Control: Water 	Swab	10	100% -	
Saliva (Fresh, direct pipetting)	None (direct sampling)	10	100% +	
Saliva stain	Paper	10	80% + (20% faint)	
Saliva-containing swab(Fresh) (At 36°C)	Swab	23	90% + (10% faint)	
Saliva-containing swab (RT)	Swab	10	100% +	

Saliva (Rim of a used glass)	Swab	10	60% + (40% faint)	
Saliva (Rim of a used glass)	None (direct sampling)	10	100%+	
Saliva stain (Fresh, neat) 	Cloth	10	100% +	
Saliva stain (Dried, neat) 	Cloth	10	100% +	
Mixture of stains: Fresh (saliva, blood, seminal fluid, & water) 	Cloth	10	100% +	
Mixture of stains: Dried (saliva, blood, seminal fluid, & water) 	Cloth	10	100% +	

*Table 1: Summary of possible positive results of various textile stains on AmylasePaper, and on the AmylaseTest*

**Patterns of Negative Results**

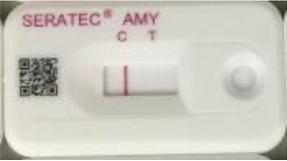
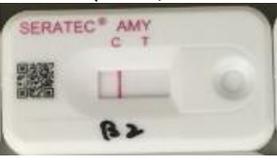
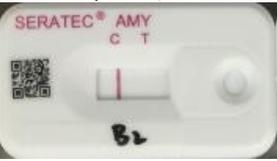
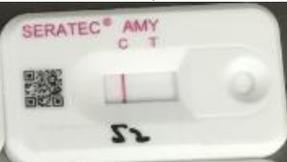
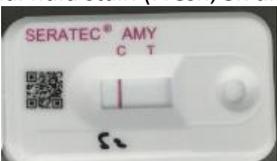
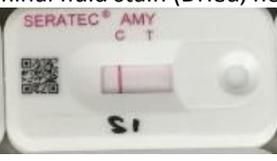
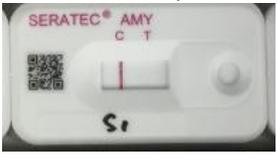
Sample Type/ AmylaseTest (protein control)	Sample Carrier	Repetitions	Average Results	Pictures
Positive control: Saliva-containing swab (RT)	Swab	10	100% +	
Water stain 	Cloth	10	100% -	
Blood stain (Dried, 100x diluted) 	Cloth	10	100% -	
Blood stain (Fresh, 100x diluted) 	Cloth	10	100% -	
Seminal fluid stain (Dried, 5x diluted) 	Cloth	10	100% -	
Seminal fluid stain (Fresh, 5x diluted) 	Cloth	10	100% -	
Seminal fluid stain (Dried, neat) 	Cloth	10	100% -	
Seminal fluid stain (Fresh, neat) 	Cloth	10	100% -	

Table 2: Summary of possible negative results of various textile stains on AmylasePaper, and on the AmylaseTest

## References

- (1) Gaensslen, R.E. 1983. Sourcebook in Forensic Serology, Immunology and Biochemistry, National Institute of Justice, 183-189.
- (2) Wurster, J.W. and Laux, D.L. 1990. A rapid amylase mapping procedure. MAFS Newsletter 19, 48-49.

## Contact Details

SERATEC® Gesellschaft für Biotechnologie mbH  
Ernst-Ruhstrat-Str. 5  
37079 Goettingen  
GERMANY

Phone: +49 (0) 551 504800

Fax: +49 (0) 551 50480-80

E-Mail: [contact@seratec.com](mailto:contact@seratec.com)

Web: [www.seratec.com](http://www.seratec.com)