Enhancing saliva detection: Dilution-Based Discrimination of α-Amylase in Saliva and Urine

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Introduction

The identification of body fluids is crucial in criminal investigations for characterizing potential biological evidence. Lateral Flow (LF) tests containing anti- α-Amylase antibodies are commonly used to presumptively detect human α-Amylase for the identification of saliva in forensic samples. When analyzing sexual assault samples, such as swabs or fabric cuttings from underwear, it is crucial to determine whether a positive result for α-Amylase is due to saliva or urine. Preliminary validations have shown that α-Amylase from urine traces is sometimes detectable at a 1:10 dilution and becomes undetectable at a 1:100 dilution, whereas salivary α-Amylase remains detectable at dilutions of at least 1:1000. These findings suggest that dilution levels can be used to differentiate the source of α-Amylase, thereby improving the accuracy of forensic analysis in sexual assault cases.

Objectives

The aim of this study is to identify the most reliable dilution ratio for detecting saliva while excluding positive α-Amylase results from urine in an LF test. Additionally, the study aims to provide standardized procedures for various extraction methods, including cuttings and swabs, to ensure accurate results in all scenarios.

Experimental setting

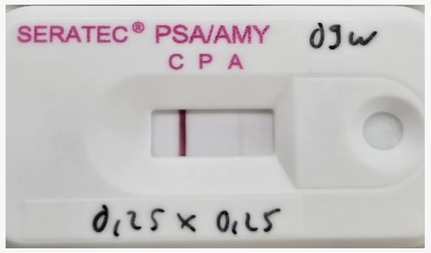
The experimental setup was designed to simulate a worst-case scenario, ensuring that any potential positive α-Amylase results due to urine could be ruled out. Urine samples from 26 individuals were tested at varying concentrations (neat, 1:10, 1:20, 1:50, 1:100, and 1:200), as well as with fully urine-soaked cotton pieces. The cotton was cut into 3x3 cm pieces for swabbing, and into 1x1 cm, 0.5x0.5 cm, and 0.25x0.25 cm pieces for direct extraction in buffer. All samples, including swabs and fabric cuttings, were extracted in 300 µL of buffer solution and applied to the SERATEC® PAM Test. Additionally, saliva samples were tested at various dilutions (1:1000, 1:2000, 1:5000, 1:7000, and 1:10,000) to establish the concentration difference in α-Amylase between urine and saliva. All experiments were performed at room temperature.

Materials and Methods

The experiments were conducted using the SERATEC® PAM Test, which detects both prostate-specific antigen (PSA) and α-Amylase, allowing for the simultaneous identification of seminal fluid and saliva in forensic samples. For swabbing, a DNA-free cotton swab (Sarstedt, REF 80.626) was used to ensure sample integrity during the experiments.

Sample dilution	Amylase positive
1:1000	10/10
1:2000	10/10
1:5000	10/10
1:7000	10/10
1:10000	9/10

Picture 1: Results of salivary α-Amylase dilution experiments



Picture 2: Example of a PAM Test with a weak positive α-Amylase result (A line) and a negative PSA result (P line)

	α-Amylase positive		α-Amylase negative	
sample	Female	male	female	male
neat urine	14	12	0	0
Urine 1:10	14	12	0	0
Urine 1:20	12	12	2	0
Urine 1:50	3	7	11	5
Urine 1:100	3	3	11	9
Urine 1:200	0	0	14	12
extr. swab	0	0	14	12
Cutting 1x1 cm	9	7	5	5
Cutting 0.5 x 0.5 cm	3	1	11	11
Cutting 0.25x0.25 cm	1	0	13	12

Picture 3: Results of diluted liquid urine samples and extracted

Results

Urine samples consistently yielded negative results at a 1:200 dilution, while α-Amylase from saliva was detectable at dilutions of up to 1:7000. Additionally, fabric pieces soaked in urine did not yield positive results for α-Amylase when swabbed. However, some fabric cuttings did produce positive results. Among the cuttings, approximately 60% of the 1x1 cm pieces tested positive for α-Amylase, compared to 15% of the 0.5x0.5 cm pieces. The smallest cuttings (0.25x0.25 cm) showed a positive result in only one instance.

Conclusion

In typical body fluid screening workflows utilizing α-Amylase LF immunoassays, a positive result attributable to urinary α-Amylase is highly unlikely. Our experiments simulated an extreme scenario using soaked fabric samples to model a worst-case condition, which is rarely encountered in practical forensic applications. Additionally, any sample should be appropriately diluted before performing an LF test, such as the SERATEC® PAM Test. To ensure accurate detection of salivary α-Amylase, we recommend the following sampling procedures: use smaller fabric cuttings (0.25 cm^2 in 300 µL of buffer) or larger cuttings (0.5 cm^2 to 1 cm^2 in 500 µL of buffer), and extract swabs in 200–500 µL of buffer, depending on the absorbed volume. Importantly, the significant difference in dilution thresholds—where urinary α-Amylase tests negative at a 1:200 dilution and salivary α-Amylase is reliably detected at a 1:7000 dilution—provides a wide margin to ensure accurate differentiation between these two sources of α-Amylase, even after dilution of suspicious samples.

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