The Detection of Amylase in DNA Extracts Using the RSID™-Saliva Test Card

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RSID™-SALIVA VALIDATION STUDY

1. Introduction

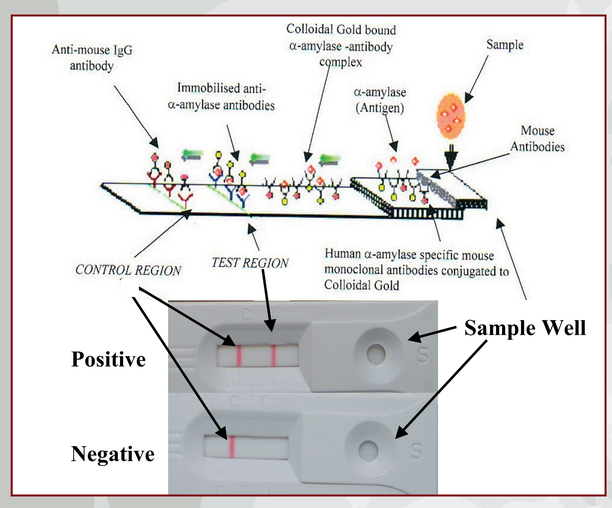
The detection of saliva in forensic cases can be a valuable tool, when used in addition to other tests, in demonstrating association between a victim and suspect. Exhibits requiring testing for saliva are often those from alleged sexual assaults and may include swabs (sampled from areas such as the neck, breasts and genitals) and clothing.

The Rapid Stain Identification, or RSID™-Saliva test, is the first immuno-chromatographic test card available for the detection of human saliva, testing for the presence, rather than the activity, of human salivary α-amylase. FSSA currently uses the SALIgAE® saliva test for identifying salivary amylase in case samples. This test is subjective, relying on the interpretation of a yellow colour change to the test solution.

The validation study for the RSID™-Saliva test investigated:

- Sensitivity
- Specificity
- Aged saliva stains
- The ability to detect saliva dilutions in blood, such as found in expirated blood stains
- RSID™-Saliva as a potential replacement method to the SALIgAE® saliva test

FIG 1: RSID™-Saliva Test Mechanism



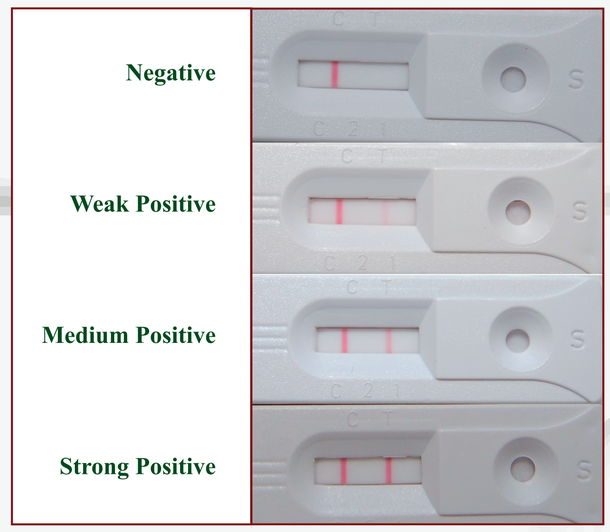
Ref: C.A Spruce, P.L Cooper, L.G Webb, A.L Borowitzka *Comparison and Validation of the SALIgAE® and RSID®-Saliva Test Kits in the Forensic Identification of Human Saliva* (2006)

2. Materials and Methods

- The RSID™-Saliva test kit includes a test protocol with separate extraction and running buffers
- A 5 mm² portion of each stain was extracted at room temperature for one hour with 200µL of RSID™ Extraction Buffer. These were centrifuged at 3,000 RPM for one minute
- 40µL of the extract was mixed with 60µL of TBS⁺ running buffer. The 100µL mixture was added directly to the RSID™-Saliva test card
- Results were recorded up to the 10 minute cut off time

For the purpose of assessing the performance of the cards during this validation, the intensity of the positive reaction was observed and grouped into the following categories:

FIG 2: Grade of Positive Reaction

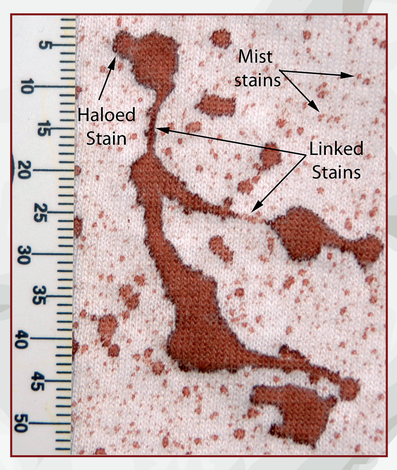


The presence of two red lines indicates the sample is positive for human salivary amylase, a red band in the control region only is indicative of a negative result.

3. Results

- The results of tests performed on wet saliva using RSID™-Saliva show that the test is capable of detecting salivary amylase to a level of 1/256 dilution, and is more reliable, more consistent and easier to use than the SALIgAE® test.
- This study has shown that the RSID™-Saliva test is specific for human salivary amylase, with no cross-reactivity observed with any of the animal saliva samples tested.
- Human faeces was the only non-saliva sample tested to produce positive results in this validation study.
- The RSID™-Saliva kit has been shown to be reliable for aged sample testing, with saliva stains up to two years of age producing positive amylase reactions.
- Amylase was detected in all expirated bloodstains tested using RSID™-Saliva (see FIGS 3 and 4).
- The RSID™-Saliva test is capable of identifying saliva recovered from a variety of substrates, even when diluted with other bodily fluids such as blood and urine.
- A number of non-biological contaminants, such as lipstick and toothpaste, failed to show any hindrance upon the RSID™-Saliva kit's ability to detect salivary amylase when testing mixed samples with saliva.

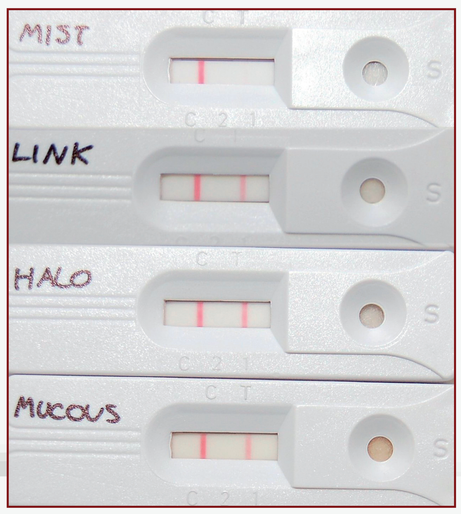
FIG 3: Expirated Bloodstains Tested Using RSID™-Saliva



Ref: R. Trönnberg, E. Silenieks, Dr. K. Both *The Recognition of Expirated Bloodstain Patterns on Cotton Fabrics* R&D Report: #R 82 (2007)

RSID™-Saliva detected amylase in all of the expirated stain types tested. The mist pattern stain produced predictably much weaker results (barely visible photographically), due to the lower levels of salivary amylase present in this stain type.

FIG 4: RSID™-Saliva Results for Expirated Bloodstains



4. Conclusion

- For recent suspected saliva stains, 5 mm² of fabric and a 1/8 portion of swabs be used for testing. For aged samples, at least 10 mm² of stained fabric should be tested.
- The RSID™-Saliva test should be used in cases when the presence or absence of human saliva will be an issue in court.
- The findings of this study showed that the RSID™-Saliva test is comparatively more sensitive and more specific than the SALIgAE® test for salivary amylase detection, and that RSID™-Saliva should replace the SALIgAE® test as a method for saliva detection and identification.
- Results found that RSID™-Saliva is a suitable method to be used for forensic casework.

COMPATIBILITY WITH DNA EXTRACTION PROCEDURES

1. Introduction

The compatibility of the RSID™ test with the DNA extraction procedure used for Profiler Plus™ was investigated. During the extraction phase, samples are initially washed with water and then centrifuged. The cellular material containing DNA forms a pellet at the bottom of the tube and the supernatant is discarded. This study investigated the ability of the RSID™-Saliva test to detect amylase in the discarded supernatant.

2. Materials and Methods

- Known saliva samples (5 buccal swabs) and 38 casework samples were assessed.
- Casework samples mostly consisted of skin swabs (from bite-marks, etc) and swabs from drink containers. It was unclear from the case information as to where the samples from the drink containers were taken from, that is whether they were saliva or contact DNA samples.
- Supernatants were collected from all samples and tested using the recommended RSID™ protocol.

3. Results (*see Table 1*)

- Amylase was detected in all known saliva samples
- 27 casework samples produced positive RSID™ results
- DNA results were obtained from all positive RSID™ samples
- 11 casework samples produced negative results
- DNA results were obtained from 6 of the 11 negative RSID™ samples
  - 4 of the 6 were skin swabs and a DNA result was to be expected
  - The remaining 2 were swabs from drink containers and it is unknown as to whether these were in fact sampled for saliva

Table 1: DNA Extract Results

Swab Sample	RSID™-Saliva Result @ 10 minutes	Reaction Time (min:sec)	DNA Result Obtained
Opening Molotov cocktail 2	Negative	-	No
Drink can 3	Negative	-	No
Cordial bottle	Negative	-	No
Pepsi bottle	Negative	-	No
Pure Blonde beer bottle	Negative	-	No
Control skin	Negative	-	Yes
Neck 3	Negative	-	Yes
Left cheek	Negative	-	Yes
Right cheek	Negative	-	Yes
Coke can 3	Negative	-	Yes
Fanta bottle	Negative	-	Yes
Lid/opening of water bottle	Weak Positive	1:30	Yes
Neck 1	Weak Positive	3:25	Yes
Neck 2	Weak Positive	3:30	Yes
Right ear	Weak Positive	5:50	Yes
Breast	Weak Positive	5:50	Yes
Drink can 1	Weak Positive	2:00	Yes
Drink can 2	Weak Positive	6:00	Yes
Drink can 4	Weak Positive	1:45	Yes
Drink can 5	Weak Positive	5:50	Yes
Coke can 1	Weak Positive	2:30	Yes
Coke can 2	Weak Positive	7:10	Yes
Beer bottle 1	Weak Positive	4:50	Yes
Beer bottle 2	Weak Positive	8:00	Yes
Black Douglas can	Weak Positive	2:30	Yes
Jim Beam can	Weak Positive	7:30	Yes
VB can	Weak Positive	3:50	Yes
Water bottle	Weak Positive	3:50	Yes
Spring water bottle	Weak Positive	5:23	Yes
Buccal swab 2	Weak Positive	2:30	Yes
Buccal swab 3	Weak Positive	3:00	Yes
Opening Molotov cocktail 1	Medium Positive	1:40	Yes
Listerine bottle	Medium Positive	1:25	Yes
Lips	Medium Positive	7:00	Yes
Upper chest	Medium Positive	2:10	Yes
Labial	Medium Positive	8:55	Yes
External vulva	Medium Positive	3:05	Yes
'Globule' of saliva on cement	Strong Positive	0:55	Yes
Neck 4	Strong Positive	0:48	Yes
Mouth of coke bottle	Strong Positive	1:10	Yes
Buccal swab 1	Strong Positive	1:50	Yes
Buccal swab 4	Strong Positive	1:50	Yes
Buccal swab 5	Strong Positive	1:50	Yes

4. Conclusion

The RSID™-Saliva test successfully detected amylase in the majority of samples. Those that produced negative RSID™ results are regarded as inconclusive, as it was not clear where the swab had been collected from and it was unknown whether these samples contained salivary amylase. It is likely then, that the RSID™-Saliva test can be used to detect salivary amylase in casework samples during the DNA extraction process, and that the RSID™-Saliva test is compatible with DNA extraction procedures.

An advantage of testing the discarded supernatant is that it minimises the amount of sample consumed for forensic testing and maximises the information obtained from that case sample.

Further work is being conducted to support the findings of this study.

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