

did the DNA come from saliva? an investigation into the detection of saliva in forensic casework using Rapid Stain Identification of Human Saliva (RSID®-Saliva)

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A question often posed in the court context for sexual offence cases where it is alleged that an oral assault has occurred is "Did the DNA come from saliva?" The validation and introduction of RSID-Saliva at the Victoria Police Forensic Services Department (VPFSD) has enabled the identification of human saliva on casework items and it can be used as a tool to help case managers answer such questions.

Independent Forensics RSID test for saliva is the first available test for the specific detection of human saliva, testing for the presence of human salivary α -amylase (Figure 1).

validation

A validation study of RSID-Saliva was undertaken testing the reproducibility, robusticity, sensitivity and specificity of RSID-Saliva using the test protocol recommended by the manufacturer¹. The findings were as follows:

- RSID-Saliva was found to give reliable, robust and reproducible results.
- RSID-Saliva can detect saliva in a range of different individuals but minimal variation was found to exist between individuals.
- Saliva can still be detected by RSID-Saliva after 5 months storage, but at a lower intensity when stored at a higher temperature. (Note: 5 months was the maximum time tested).
- RSID-Saliva gave positive results when saliva was diluted 1/128.
- No high dose hook effect was found to occur.
- RSID-Saliva showed no cross-reactivity with the saliva of any of the animals tested (Table 1)
- RSID-Saliva showed no cross-reactivity with other tested human body fluids except human breast milk (Table 2 & Figure 2)

Source of Saliva	RSID-Saliva Result
Cat	NEG
Chicken	NEG
Dog	NEG
Duck	NEG
Guinea Pig	NEG
Horse	NEG
Rabbit	NEG
Sheep	NEG

Table 1. Negative RSID-Saliva results were obtained when testing the saliva of a range of animals.

Biological Material tested	RSID-Saliva Result
Vaginal Secretions	NEG
Semen	NEG
Blood	NEG
Urine	NEG
Faeces	NEG
Sweat	NEG
Tears	NEG
Nasal Secretions	NEG
Breast Milk	POS

Table 2. Negative RSID-Saliva results were obtained when testing human body fluids, except human breast milk.

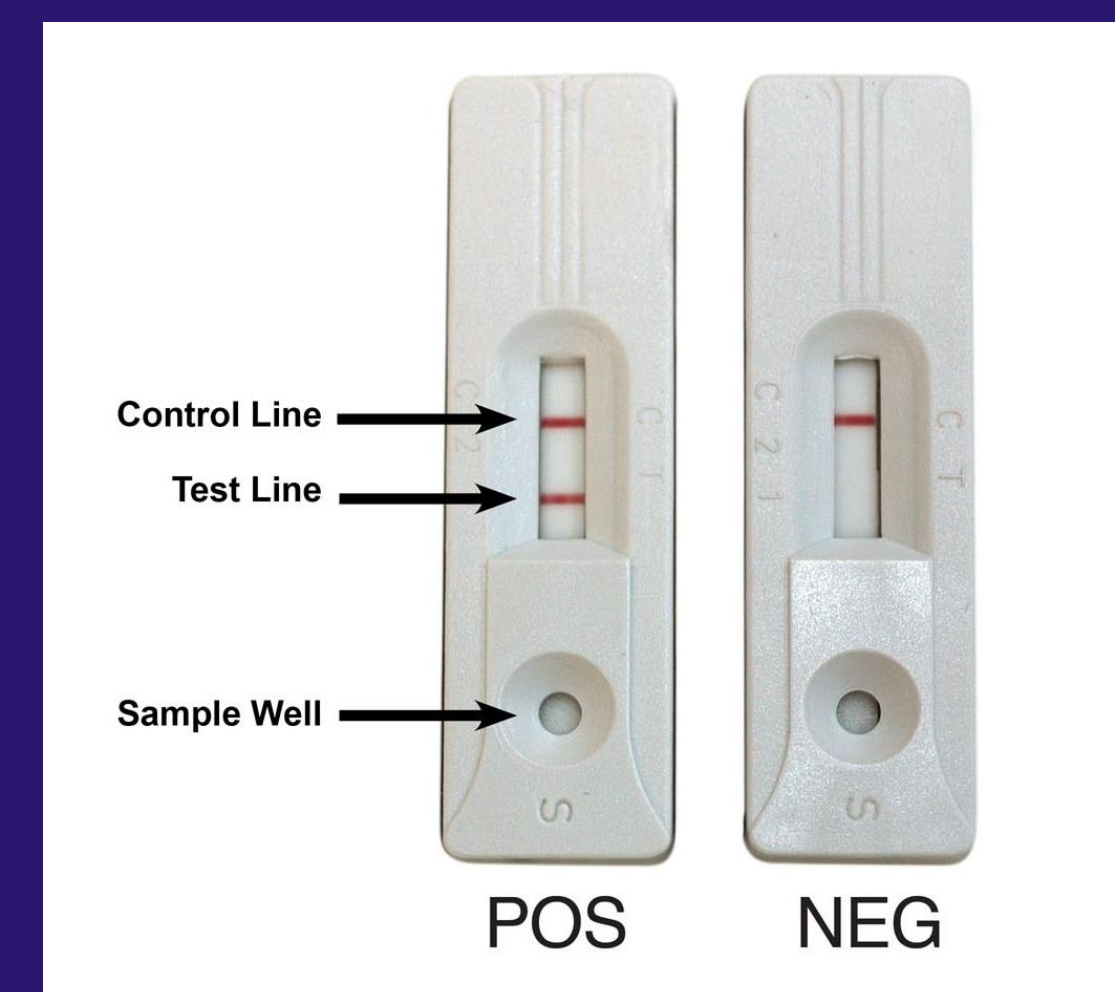


Figure 1. The test devices show a positive and negative result representing the presence and absence of human saliva respectively. The test also has a built in control to ensure that the test has worked correctly.

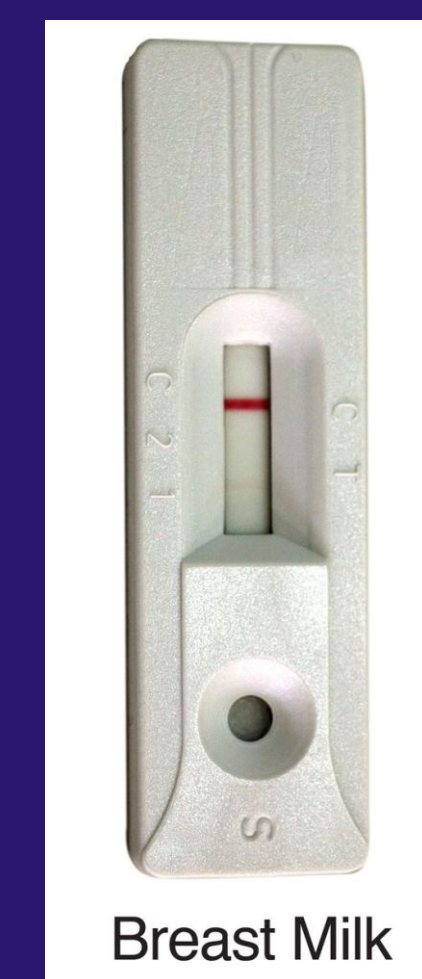


Figure 2. A weak positive RSID-Saliva result was obtained from human breast milk.



simulated casework scenarios

Studies were also conducted examining the retrieval and detection of saliva using RSID-Saliva in a range of simulated casework scenarios and the integration of such tests into DNA analysis at the VPFSD:



- Saliva could be detected with RSID-Saliva on swabs from the skin of a normal active individual 72 hours post saliva deposition. After DNA analysis of the swabs, mixed DNA profiles were obtained that could be accounted for by the donor of the saliva and the individual that the saliva was swabbed from. Note: the DNA originating from the saliva may not necessarily be in the majority of a mixed DNA profile when both saliva and skin cells are present (Figure 3).
- A positive RSID-Saliva result was still obtained from swabs of the skin that had been deposited with saliva even after the skin had been wet prior to swabbing.
- Positive RSID-Saliva results were obtained from penile and vulval swabs after oral intercourse.
- Positive RSID-Saliva results were obtained from a condom that had saliva on it.
- A positive RSID-Saliva result was obtained from the mouth area of a water bottle that had been drunk from. However, a negative RSID-Saliva result was obtained from a beer bottle.
- RSID-Saliva can detect saliva that has been deposited onto different highly dyed materials and DNA profiles were obtained from the material that matched the profile of the donor of the saliva.
- RSID-Saliva can detect saliva on clothing after it was first subjected to tape lifting, whether saliva was deposited directly onto clothing or if it was deposited by secondary transfer events. Therefore, to maximise the chance of getting a DNA result, it is recommended that clothing suspected to contain saliva is sampled for DNA via tape lifting prior to the RSID-Saliva test.

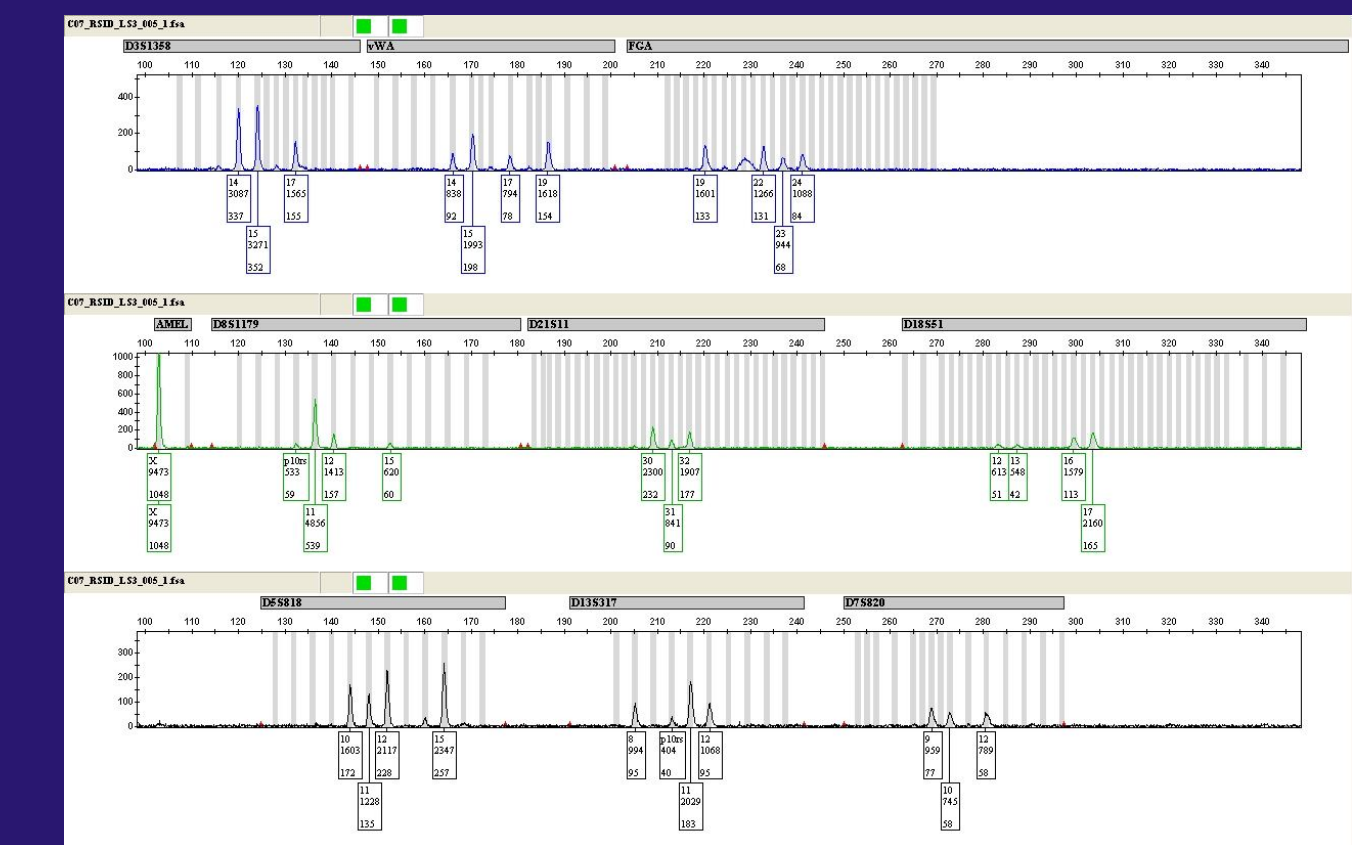


Figure 3. Mixed DNA profile obtained from a swab of skin 11 hours after saliva deposition. The DNA originating from the skin cells is in the majority compared to the DNA originating from the saliva.

- If the presence of semen as well as saliva is in question, saliva can be detected on clothing with RSID-Saliva after it was first subjected to the Acid Phosphatase method.
- The polilight was found to be a useful aid to locate areas on clothing that could be tested with RSID-Saliva.

secondary transfer studies

A detailed study into the detection of saliva with RSID-Saliva after secondary transfer events simulating possible casework scenarios was also conducted as follows:

Saliva was deposited onto the skin of an individual and left to dry for various time periods (t = 0, 30 & 60 minutes) before being contacted moderately with material for approximately 10 seconds. (At t = 60, the saliva on the skin was dry). The piece of material was then tested with RSID-Saliva.

- Positive RSID-Saliva results were obtained from the pieces of material at all time intervals tested. This indicates that saliva could be transferred from the skin to clothing and detected by RSID-Saliva, even after it had first dried on the skin.
- Some saliva still remained on the skin after contact with the clothing using RSID-Saliva. Such that, in casework it may be relevant to examine the victims clothing that has been in contact with an area of the skin suspected to contain saliva of the offender, as well as swabs of the area of the skin.

Saliva was deposited onto a piece of material and left to dry for various time periods (t = 0 & 60 minutes) before being contacted moderately with a secondary piece of material for approximately 10 seconds. The secondary piece of material was then tested with RSID-Saliva.

- Positive RSID-Saliva results were only obtained from the secondary piece of material at t = 0 and negative RSID-Saliva results were obtained when t = 60. This indicates that either saliva did not transfer from material to material when it was dry (at t = 60) or it could not be detected with RSID-Saliva on the secondary piece of material. However, when the saliva was wet (at t = 0), the saliva did transfer between the two pieces of material and could be detected by RSID-Saliva.

The RSID-Saliva test was found to be a fast, easy to use, reliable and valid method for the identification of human saliva. From the results of this validation study and the ability to detect saliva using RSID-Saliva in typical casework scenarios even after secondary transfer events; and the fact that the test can be integrated into DNA analysis; the RSID-Saliva test has been adopted for use at the VPFSD for human saliva identification.

use in casework

The use of RSID-Saliva has shown to be a valuable tool in the following types of forensic casework at the VPFSD:

- Examination of a complainant's jean for saliva transfer and DNA after it was alleged that a doctor kissed her thigh during a routine medical examination. (Note: Thigh swabs were not collected).
- Examination of a male child's pyjama pants for saliva transfer and DNA after it was alleged that an offender performed oral intercourse on him. (Note: Penile swabs of the complainant were examined prior to the introduction of RSID-Saliva. A DNA profile was obtained from which the complainant and the accused cannot be excluded from. The presence of DNA from the accused could be explained by 'touch' DNA).
- Examination of a complainant's clothing for saliva and DNA corresponding to bite marks after it was alleged that she was bitten through her clothing during a rape.
- Examination of a step daughter's breast swabs and step father's underpants for saliva and DNA after it was alleged that the step daughter was made to perform oral intercourse on her step father and he licked her breasts.



references

1. Independent Forensics. Rapid Stain Identification of Human Saliva (RSID®-Saliva) Provided Protocols (April 2007)

