Identification of Human Saliva:

A comparison of the SALIgAE® test and the Rapid Stain Identification (RSID®) – Saliva test kit.

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The detection of saliva in forensic cases can be a valuable tool, when used in addition to other tests, to demonstrate association between a victim and suspect. Exhibits requiring testing for saliva are usually those requested from alleged oral assaults and may include swabs (sampled from such areas as the neck, breasts and genitals) and clothing. The detection of saliva can also be used as a screening test to determine areas most likely to contain a high concentration of buccal cells (1). Two commercial kits that are currently available are the Abacus Diagnostics SALIgAE® Test for the Forensic Identification of Saliva and the Rapid Stain Identification (RSID®)-Saliva test kit.

The SALIgAE® Test for the Forensic Identification of Saliva kit allows for the detection of saliva. According to the manufacturer, this test kit has the ability to detect trace amounts of saliva and offers a higher level of sensitivity and specificity when compared to that of the Phadebas® Amylase test. In the presence of saliva, a reaction occurs with the colourless reagent contained within the test vials, producing a bright yellow colour change. The manufacturer has not yet disclosed the reaction mechanism of the SALIgAE® Saliva test (2,4).

The Rapid Stain Identification (RSID®) test for saliva is the first available test kit for the specific detection of human saliva, testing for the presence of human salivary α-amylase (Figure 1). The test is comprised of immunochromatographic strips that use two mouse monoclonal antibodies specific for human α-amylase; one form of antibody is conjugated to colloidal gold present in the sample pad of the kit, whilst the other form of antibody is present in strips on the test region of the kit. If human α-amylase is present in the test sample when it is added to the kit, an antigen-antibody conjugated to a colloidal gold complex will form. As this complex migrates down the kit test substrate, the immobilised anti-α-amylase antibodies on the test region bind the α-amylase -antibody-gold complexes, producing a red coloured band. The kit also contains an internal control consisting of anti-mouse IgG antibody. The anti-mouse IgG on the control region binds any mouse antibodies migrating past the control region, producing a red band (3).

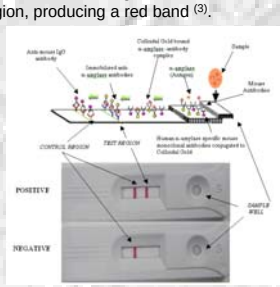

Figure 1: Positive and Negative results of the RSID®-Saliva test kit (adapted from (3))

The sensitivity and specificity of both kits were compared to assess their suitability as a confirmatory test in the forensic detection of human saliva. Sensitivity was tested using a serial dilution of human saliva and specificity was tested using a range of bodily fluids other than saliva, animal saliva and substances and substrates that are likely to be mixed with saliva on forensic exhibits.

METHODS

SALIgAE® testing was conducted according to the supplied Technical Information Sheet (Rev 5/05) (2).

RSID®-Saliva testing was conducted according to the supplied Provided Protocols (Rev. B 2006) (3), which was prior to the release of the Extraction Buffer being supplied with the kit. Phosphate Buffered Saline (pH 7.4) was used as the extraction buffer for all testing, excluding the tests conducted on using sterile purified water as the extraction buffer (see sensitivity results).

RESULTS

Scale of Reactivity	Intensity of Yellow Colour Change
NEG	No Colour Change
1	Hint of Colour (Trace)
2	Very Weakly Visible
3	Weakly Visible
4	Visible
5	Clearly Visible Strong Yellow Colour

Table 1: SALIgAE® Scale of Reactivity

SALIgAE® results were determined using a "Scale of Reactivity" grading system of the intensity of the yellow colour produced. This grading system was based on studies performed by Carlesso *et.al.,* (2005)(4) (Table 1).

RSID®-Saliva results were read according to the Provided Protocol (Rev. B 2006) (3) (Table 2).

Result	Bands Present
POSITIVE	2 bands present : 1 in the test region, 1 in the control region
NEGATIVE	1 band present in the control region only
INVALID	No bands present, or 1 band in the test region only

Table 2: RSID®-Saliva Results

SENSITIVITY

The SALIgAE® test was more sensitive overall than the RSID®-Saliva test, with a dilution end point range of 1:128-256, as opposed to the RSID®-Saliva dilution end point of 1:2-128 (Tables 3 & 4).

	Male Saliva Donor 1		Female Saliva Donor 1	
	SALIgAE® Result	RSID®-Saliva Result	SALIgAE® Result	RSID®-Saliva Result
Dilution		PBS H_2O		PBS H_2O
NEAT	5	POS POS	5	POS POS
1/2	5	POS POS	5	POS POS
1/8	4	POS POS	4	POS POS
1/32	3	POS NEG	3	POS POS
1:128	1	NEG NEG	1	POS POS
1:256	NEG	NEG NEG	NEG	NEG NEG
1:512	NEG	NEG NEG	NEG	NEG NEG
1:1024	NEG	NEG NEG	NEG	NEG NEG
1:2048	NEG	NEG NEG	NEG	NEG NEG
1:4096	NEG	NEG NEG	NEG	NEG NEG

Table 3: Sensitivity Testing

	Male Saliva Donor 2		Female Saliva Donor 2	
	SALIgAE® Result	RSID®-Saliva Result	SALIgAE® Result	RSID®-Saliva Result
Dilution		PBS H_2O		PBS H_2O
NEAT	5	POS POS	5	POS POS
1/2	4	POS POS	4	POS POS
1/8	3	POS POS	3	POS POS
1/32	2	POS POS	2	POS POS
1:128	1	NEG POS	1	POS POS
1:256	1	NEG NEG	1	NEG NEG
1:512	NEG	NEG NEG	NEG	NEG NEG
1:1024	NEG	NEG NEG	NEG	NEG NEG
1:2048	NEG	NEG NEG	NEG	NEG NEG
1:4096	NEG	NEG NEG	NEG	NEG NEG

Table 4: Sensitivity Testing (cont'd)

To ensure that routine use of RSID®-Saliva testing would be compatible with laboratory DNA extraction methods, results from extraction of saliva in water over time periods of 10 minutes and 90 minutes were compared to the extraction of saliva in PBS at the same time periods. DNA profiling was also conducted to ensure that the extraction medium and time period did not have a negative effect on the DNA profile obtained.

No significant differences were noted for extraction mediums, times and DNA yield (Figures 2 & 3).

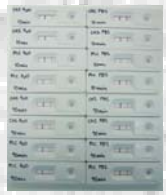

Figure 2: Results obtained from samples extracted at 10 minute period and 90 minute period in either PBS or sterile purified water.

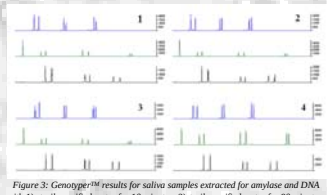

Figure 3: Genotyper™ results for saliva samples extracted for amylase and DNA with 1) sterile purified water for 10 minutes; 2)sterile purified water for 90 minutes; 3) PBS for 90 minutes and; 4) PBS for 10 minutes

SPECIFICITY

False negatives were not observed using RSID®-Saliva (Table 5).

False negatives were not observed using SALIgAE® , however coloured substances (e.g. blood) did mask the colour change reaction in the SALIgAE® test. On the case of the blood mixed with saliva, results appeared more orange in colour than the blood controls, indicating that there was yellow colour change result. The intensity of this yellow colour change results however, could not be determined (Figure 4).

Figure 4: Colour results observed for blood (male and female) control samples and blood (male and female) mixed with saliva.

SAMPLE	SALIgAE® RESULT	RSID® Saliva RESULT
Male Blood	2ND/ET	NEG
Male Blood + Saliva	2ND/ET	POS
Female Blood	2ND/ET	NEG
Female Blood + Saliva	2ND/ET	POS
Male Sweat	NEG	NEG
Male Sweat + Saliva	4	POS
Female Sweat	NEG	NEG
Female Sweat + Saliva	4	POS
Semen	NEG	NEG
Semen + Saliva	4	POS
Vaginal Secretions	NEG	NEG
Vaginal Secretions + Saliva	4	POS
Male Urine	1	NEG
Male Urine + Saliva	4	POS
Female Urine	2	NEG
Female Urine + Saliva	4	POS
Male Faeces	NEG	NEG
Male Faeces + Saliva	5	POS
Female Faeces	NEG	NEG
Female Faeces + Saliva	5	POS
Breast Milk	3	POS
Breast Milk + Saliva	4	POS
Expired Blood	2ND/ET	POS

Table 5: Specificity testing

False positive results were obtained for the human breast milk controls using both test kits (Figures 5 & 6).

Figure 5: Colour results observed for urine (male and female) control samples and urine (male and female) mixed with saliva.

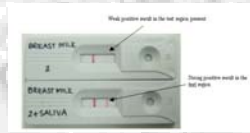

Figure 6: Weak false positive result obtained for the breast milk control. Strong positive result obtained for the breast milk mixed with saliva.

False negative and false positive results were not observed with either test kit for the animal saliva tested (Table 6).

	SALIgAE®	RSID®-Saliva
Canine (Dog)	NEG	NEG
Feline (Cat)	NEG	NEG
Equine (Horse)	NEG	NEG

Table 6: Animal saliva specificity testing

	SALIgAE®	RSID®-Saliva
Mouthwash	NEG	NEG
Mouthwash + Saliva	5	POS
Toothpaste	NEG	NEG
Toothpaste + Saliva	4	POS
Lipstick	NEG	NEG
Lipstick + Saliva	5	POS
Perfume	NEG	NEG
Perfume + Saliva	4	POS

Table 7: Substance specificity and interference testing

	SALIgAE®	RSID®-Saliva
Cigarette Butt	1	NEG
Cigarette Butt + Saliva	1	POS
Aluminium Can 1	NEG	NEG
Aluminium Can 1 + Saliva	1	NEG
Envelope	NEG	NEG
Envelope + Saliva	1	POS
Stamp	NEG	NEG
Stamp + Saliva	3	POS
Black Denim	NEG	NEG
Black Denim + Saliva	5	POS
Blue Denim	NEG	NEG
Blue Denim + Saliva	5	POS
Nylon	NEG	NEG
Nylon + Saliva	5	POS
Cotton	NEG	NEG
Cotton + Saliva	5	POS
Skin Swab (no saliva)	NEG	NEG
Skin Swab (licked skin)	1	NEG

Table 8: Substrate specificity and interference testing

False negative and false positive results were not observed in either test kit for any of the substrate and substance controls tested. The leaching of the tobacco from the cigarette butt during the extraction process explains the yellow coloured solution obtained from the cigarette butt using SALIgAE® (Table 8).

Varying results were obtained for saliva in the substances and substrates tests using RSID®-Saliva. The concentration of saliva recovered from these substances and substrates was most likely past the dilution end point observed for RSID®-Saliva testing (Table 8).

ENVIRONMENTAL CONDITIONS

Subjecting the saliva samples to various environmental conditions (listed in Table 9) had no effect on the results obtained using SALIgAE®, however varying results were obtained using RSID®-Saliva .

	SALIgAE®	RSID®-Saliva SET 1	RSID®-Saliva SET 2
7 days @ 37°C	5	NEG	POS
7 days @ 56°C	2	POS	NEG
7 days @ -20°C	5	POS	POS
10 minutes in UV Cabinet	5	POS	NT

NT = Not Tested

Table 9: Environmental Testing Conditions

CONCLUSIONS

Sensitivity was comparable using SALIgAE® and RSID®-Saliva, however, a slightly higher sensitivity was observed for both male and female saliva samples using the SALIgAE® kit.

No high dose hook effect was observed using the SALIgAE® or RSID®- Saliva kits.

The same level of specificity was observed in the SALIgAE® kit and the RSID®-Saliva kit, however, there was difficulty in determining the intensity of the colour change result produced by the SALIgAE® kit when the test samples were coloured themselves e.g. blood, urine and cigarette butts.

The RSID®-Saliva kit was considerably easier to use than the SALIgAE® test kit and the RSID®-Saliva kit also has an inbuilt positive control so the operator can be certain that the test has worked and the results obtained are reliable.

REFERENCES

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