Locating Saliva Stains using the Polilight® and SALIgAE® Spray

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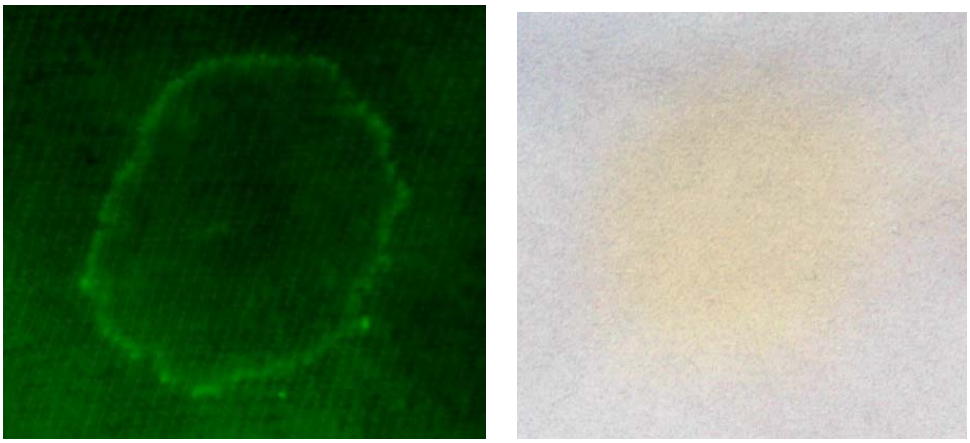

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CONTENTS

1. INTRODUCTION

In recent years the impact of trace DNA has reduced the demand for detection of saliva in forensic casework. However where trace DNA can only prove contact between persons, saliva identification can prove that a significant and in some cases malicious contact has occurred, and thus carries more evidentiary weight. Recently the *SALIgAE® Test for the Forensic Identification of Saliva* has been validated as a suitable test for indicating the presence of salivary amylase in swabs or stains identified on case items. The SALIgAE® Test however is limited in that it is a vial test, and requires an extraction step that effectively prevents its use as a screening tool for locating saliva stains on case items. The current Spotty Paper Test, based on the Phadebas® Amylase test, ceased production in December 2005, with FSSA current stocks expiring at the end of June 2006. Consequently a suitable replacement for the Spotty Paper test is required for the detection of saliva stains on case items.

Abacus Diagnostics Incorporated, who produces the *SALIgAE® Test for the Forensic Identification of Saliva*, have developed a new screening test, the *SALIgAE® Spray for the Location of Saliva Stains at the Crime Scene*. The SALIgAE spray contains the same test reagent as the SALIgAE vial test. It is envisaged that the SALIgAE® Spray will be used in a similar way as the Spotty Paper Test to provide the capacity for screening of case items for saliva. The manufacturer claims that the SALIgAE® Spray Test is stable, simple to use, requires no reagent preparation and provides rapid results in ten minutes (Abacus Diagnostics 2005).The manufacturer also highlights that, as compared with the Spotty Paper test, the SALIgAE® Spray requires no temperature incubation step and no additional equipment.

The SALIgAE® Spray Test works in a similar fashion to the old Spotty Paper Test, whereby the suspected saliva stain is pressed to transfer a portion of the stain to a moistened test paper. The difference is however that instead of the impregnated Spotty paper being pressed onto the item for 45 minutes, a filter paper moistened with water is pressed onto the area, much the same as the Seminal Acid Phosphatase Test. This filter

paper pressing is then sprayed with the SALIgAE® Spray reagent and results read up to a maximum time of ten minutes (Abacus Diagnostics 2005). To date, Abacus Diagnostics Incorporated has not released the mechanism of the SALIgAE® reaction, however it is known that a chemical reaction involving salivary amylase and the colourless SALIgAE® reagents produces a yellow colour change. A Summer Student Project report titled *The SALIgAE® Test for the Forensic Identification of Saliva* revealed the SALIgAE® Test to have a level of sensitivity of saliva detection suitable for forensic casework (Carlesso, Silenieks et al. 2005).

When dried, saliva stains are virtually colourless and difficult to detect. Some literature sources state that saliva stains on clothing can be detected using alternate light sources such as the using the Polilight® (Vandenberg and Oorschot 2006). The Polilight®, manufactured by Rofin Australia, is an alternate light source used widely in forensic casework. A main feature of the Polilight® is its tuneable UV and visible band pass filters that allow visualisation of fluorescent samples. These filters are used in conjunction with coloured goggles or interference filters of higher wavelengths that block out the incident light to reveal fluorescence. This feature has proven especially effective in locating semen stains on forensic casework items due to their strong fluorescence.

The aim of this research and development project was to develop a suitable method for the screening of forensic casework items for saliva stains by:

1. Assessing the visual and physical characteristics of saliva stains on different fabrics.
2. Determining whether saliva stains can be located by fluorescence methods using the Polilight®.
3. Validating the new *SALIgAE® Spray for the Location of Saliva Stains at the Crime Scene* for screening forensic casework items for the presence of suspected saliva stains.

2. MATERIALS AND METHOD

2.1 Preparation of Stains

2.1.1 Saliva Dilutions

To study the effect of stain dilution on Polilight® and SALIgAE® Spray screening, fresh pooled saliva was diluted 1 in 2, 1 in 4, 1 in 8 and 1 in 16 in Phosphate Buffered Saline (PBS). 100µL of fresh neat saliva and 100µL of each dilution was applied to sections of white unwashed cotton and allowed to air-dry for 24 hours. Five stains, each of the neat saliva and the 4 dilutions, were prepared.

2.1.2 Saliva on Different Material Types

Saliva was collected from several persons into sterile containers. All samples were pooled to provide a standard stock solution and eliminate possible variations in amylase levels (Kipps and Whitehead 1975). Stains were made from the pooled saliva on the day of collection.

To assess fabric composition and colour/dye effects on saliva detection a variety of clothing in different colours and blends were collected. The 5 categories investigated were 100% cotton, polyester/cotton blend, 100% polyester, fleecy and denim, each in the colours white, pale to medium blue, red and black or very dark, and also patterned items. A total of 28 articles of clothing were used in this study, as summarised in Table 2-1.

Material Type	Colour	Description
Cotton	White Pale-blue Red Black Pattern (white/ blue/brown/grey)	T-shirt Singlet T-shirt Singlet Shirt
Polyester/Cotton	White Pale-blue/navy/white Red Black Pattern (navy/blue check)	Shirt Zip-up vest Shorts Bike-shorts Pants
Polyester	White Blue Red Black Pattern (blue/white) Pattern (dark grey/grey)	Shorts Blouse Pants Sheer Skirt Skirt Boxer Shorts
Fleecy	White Blue Red Dark Navy Pattern (green/red/yellow/blue) Purple	Jumper Jumper Jumper Tracksuit Pants Jumper Tracksuit Pants
Denim	White Light Blue (worn) Blue (worn) Red Dark Blue (new looking) Black	Jeans Jeans Jeans Jeans Jeans Skirt

Table 2-1: Summary of Fabric Types and Colours

Each item was screened with the Polilight® using a range of wavelength/interference filter combinations to identify areas of contaminant staining already present. Any areas of soiling or staining that could interfere with saliva detection were avoided.

Two grid patterns of 6cm x 6cm squares were drawn onto each item of clothing as shown in Figure 1. 100µL undiluted saliva was applied into the first box using an auto-pipette and 100µL of 1 in 4 dilution applied to the second of the three boxes (Based on results in 3.1.1, 1 in 4 dilution was used). A cotton swab was used to smear saliva into the third box of the grid (Figure 2-1). All stains were allowed to air-dry overnight before being viewed using the Polilight®. All stains were tested with the SALIgAE® Spray after 5 days.

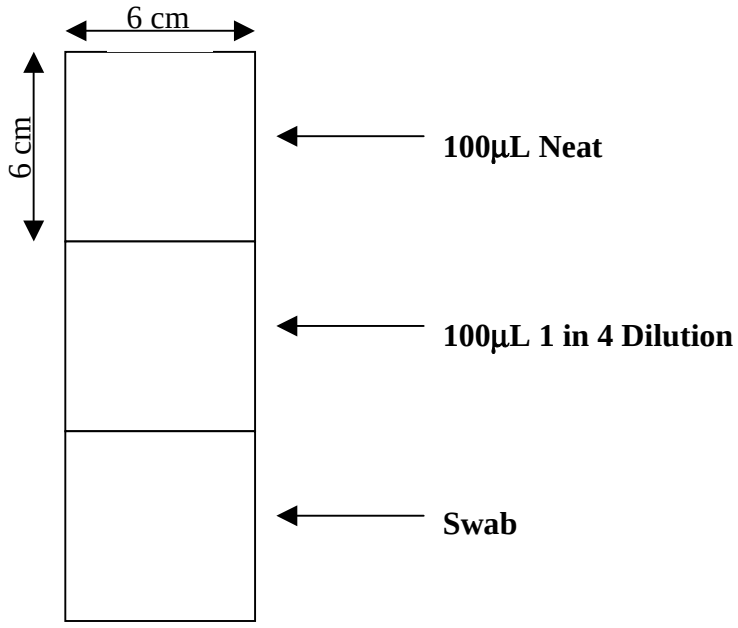

Figure 2-1: Diagram of saliva stain application on 28 articles of clothing.

2.1.3 Mock Exhibits

Mock exhibits were generated to investigate detection of saliva stains with the Polilight® and SALIgAE® Spray. Several items were selected from the 28 articles of clothing for application of a larger volume of saliva, specifically some fabric types that had proven troublesome for Polilight® detection (as per results in 3.1.2). Saliva was deposited directly from the mouth onto the clothing and the coordinates of each stain recorded using axes drawn onto the fabric. All stains were allowed to air-dry overnight before Polilight® screening.

Fabric gags made from a white cotton singlet, a blue and white polyester/cotton check shirt, blue, yellow and white acrylic/nylon football socks and strips of polyester satin in purple and maroon. The cotton singlet and polyester/cotton shirt were each cut to create long strips. The cotton strip, maroon satin strip and one of the socks were worn as gags inside the mouth for a total of 10 minutes, while the polyester/cotton strip and purple satin were worn covering the mouth also for 10 minutes. All gags were allowed to air-dry overnight.

A pair of black nylon/elastane pantyhose were cut to create two facemasks. Each of the pantyhose-leg masks were worn over the face as a mask for a period of 10 minutes and allowed to air-dry overnight.

2.1.4 Saliva and Other Stains

Additional saliva stains were made on washed white cotton as well as stains of other liquids to investigate their appearance. Saliva was applied directly by mouth to sections of boiled cotton and 'cold power' washed cotton. Water, amylase, urine, apple juice, phosphate buffered saline (PBS), sodium hypochlorite, black tea and 'Spray n' Wipe' brand cleaner were applied to sections of white unwashed cotton. All stains were allowed to air-dry overnight.

2.2 Polilight® Location of Saliva Stains

To determine optimum screening conditions for the various fabrics, different combinations of filters and goggles were trialled. All Polilight® observations were made using the PL 500 model Polilight®.

2.2.1 Saliva Dilutions

Diluted saliva stains on unwashed white cotton were viewed using the Polilight® at 470nm with the 555nm interference filters. All diluted stains were compared with a neat stain to measure relative fluorescence intensity.

2.2.2 Saliva on Different Material Types

To determine whether saliva could be located visually and with the Polilight®, each of the neat, 1 in 4 dilution and smear stains on the 28 articles of clothing were screened in natural light and then examined with the Polilight®, using different combinations of wavelengths and interference filters. The appearance of each stain was rated based on the grading system as follows, with examples shown in Figure 2-2:

0	No fluorescence detectable
1	Very weak fluorescence, barely visible
2	Weak fluorescence
R	Glue-like appearance visible with naked eye

Due to the weak nature of saliva fluorescence a grading system from 0-2 was adopted instead of the usual 0–4 grading system (Williams, Silenieks et al. 2004). In addition the rating 'R' was used to indicate visibility with the naked eye in a 'glue-like' stain.

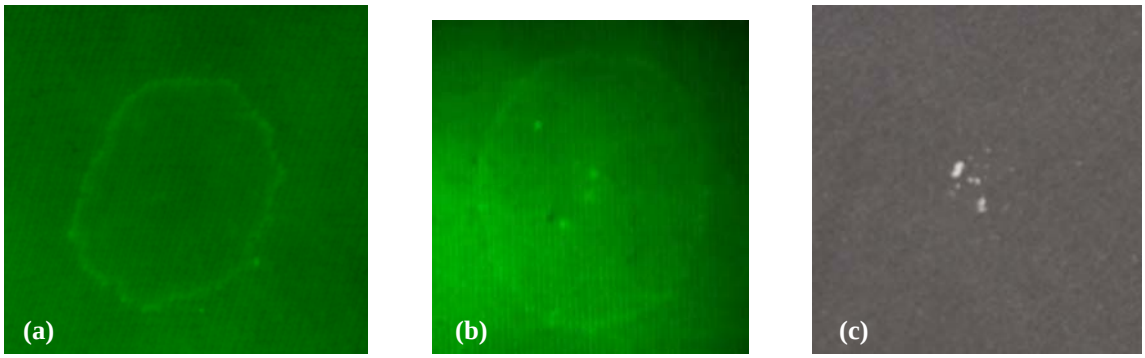

Figure 2-2: Visual Examples of the Fluorescence Grading System (a) rating '2' for weak fluorescence, (b) rating '1' for very weak fluorescence and (c) the visual rating 'R'.

2.2.3 Mock Exhibits

The mock exhibits with a larger volume of saliva were examined with the Polilight® using the excitation wavelength and filter combination deemed optimum for the specific fabric type, as per results in 3.1.2. Stains were given a rating according to the grading

system outlined in 2.2.2. Stains were also examined with the Polilight® by another person not involved in their application, in order to simulate the searching of evidence samples for saliva.

Mouth gags were examined with the Polilight® using different combinations of excitation wavelength and interference filters and stains were rated according to the grading system outlined in 2.2.2.

2.2.4 Saliva and Other Stains

Water, urine, apple juice, PBS, sodium hypochlorite, black tea and 'Spray n' Wipe' brand cleaner stains, plus saliva stains on boiled and 'cold power' washed white cotton were examined in natural light and then using the Polilight® at excitation wavelength 470nm with 555nm and 530nm interference filters and at excitation wavelength 450nm with 555nm interference filters. Fluorescence intensity and appearance were investigated relative to an untreated, neat saliva stain.

2.3 Detection with SALIgAE® Spray

2.3.1 SALIgAE® Spray Method

16 vials each of 2 formulations of SALIgAE® Spray were donated by Abacus Diagnostics International and are referred to as Formulation 1 and Formulation 2 respectively:

Formulation 1:	Catalog #900001	**Formulation 2:**	Catalog #993297
	Lot: 6632032706A		Lot: 5632021606B
	Expires JUN – 2006		Expires JUN – 2006

The SALIgAE® Spray testing protocol was followed as outlined by Abacus Diagnostics Inc. (Abacus Diagnostics 2005). Whatman no. 1 filter papers were moistened with de-ionised water and pressed over stains in the set-up as shown in Figure 2-3. Over the course of the experiments, different pressing masses were applied and are specified in each case. The moistened filter paper was pressed onto a stained area for 5 minutes

before being sprayed with SALIgAE® Spray. The filter paper was then sealed in a zip-lock bag to prevent drying during colour development. A positive result was indicated by a yellow colour change. Time for positive reaction to occur was recorded up to the manufacturer's cut-off time of 10 minutes. A negative result was indicated by no colour change.

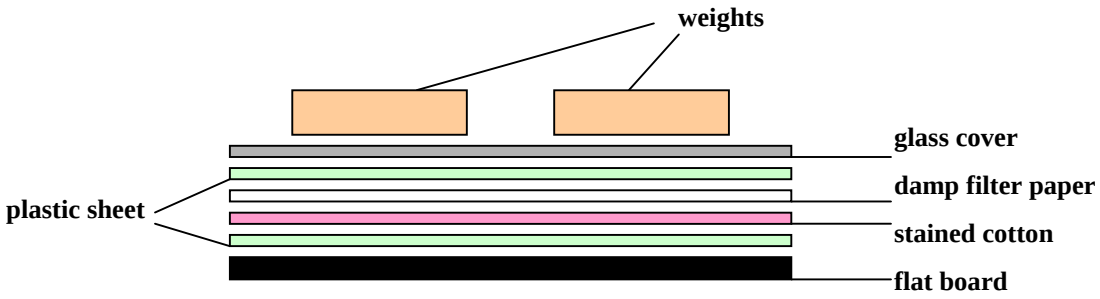

Figure 2-3: Schematic of SALIgAE® Spray pressing set-up showing layers used

2.3.2 Saliva Dilutions on White Cotton

SALIgAE® Spray testing was conducted as outlined in 2.3.1. Neat saliva stains and dilutions of 1 in 2, 1 in 4, 1 in 8, 1 in 16 and negative controls were tested in duplicate using both formulations of SALIgAE® Spray. Approximately 5kg of weight was used to press each set of 5 stains and the time taken for colour development was recorded up to the 10-minute cut-off time. Details of any colour development occurring beyond this cut-off time were also recorded.

2.3.3 Saliva on Different Material Types

SALIgAE® Spray testing was conducted as outlined in 2.3.1. Approximately 5kg of weight was used for each test. At least one item of clothing in each category of fabric type was pressed using a total weight of approximately 10kg in order to investigate the

effect of additional weight on development time and intensity. The number of 'pumps' of SALIgAE® Spray used was also varied to determine optimum spray volume.

2.3.4 Mock Exhibits

The larger volume mock exhibits were not tested with SALIgAE® Spray.

All of the fabric gags and the two pantyhose facemasks were tested with SALIgAE® Spray as outlined in 2.3.1. All items were pressed with 5.5kg weights. Either formulation of SALIgAE® Spray was used to test the gags as no significant difference had been found between the two. Yellow colour development after the 10-minute period was not monitored.

2.4 Spotty Paper Testing

Selected saliva stains were also tested with the Spotty Paper Test for comparative purposes. Items selected were saliva dilutions on white cotton from 2.1.2 and one set of stains from each fabric category in 2.1.3 including the black cotton singlet, patterned polyester/cotton pants, red polyester pants, dark fleecy tracksuit pants and black denim skirt. The standard Spotty Paper Testing protocol was followed, as outlined in Appendix 2. Areas tested with Spotty Paper had all been previously tested using SALIgAE® Spray. Results were recorded as positive, weak positive, negative (trace) and negative.

3. RESULTS

3.1 Polilight® Detection

3.1.1 Saliva on White Cotton

Optimum contrast of saliva stains on white cotton background was achieved using the 470nm excitation wavelength with the 555nm interference filters. Effective contrast was also achieved using 470nm excitation and 530nm interference filters, and the 450nm excitation with 555nm interference filters. Saliva stains were circular in shape and fluorescence was concentrated around the rim of the stains but not present in the centre. Saliva stains were found to be of a low intensity as compared with semen stains on identical material as shown in Figure 3-1.

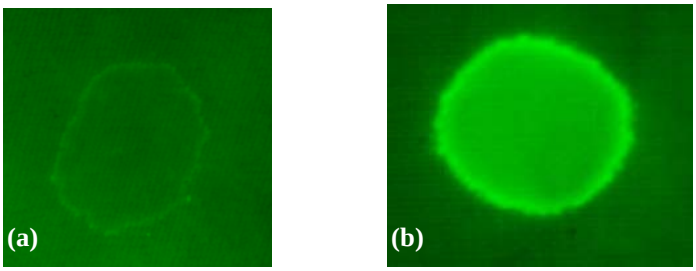

Figure 3-1: Fluorescence of (a) saliva stain on cotton and (b) semen stain on cotton

Each of the diluted saliva stains were detectable using the Polilight® at 470nm with the 555nm interference filters. No difference in fluorescence intensity was observed between the neat and the diluted stains. Neat saliva stains on white cotton that had been frozen for 5 days appeared identical to control stains.

3.1.2 Saliva on Different Material Types

See Table 7.1

Neat Stains

Saliva stains were found to have very weak fluorescence and consequently were very difficult to detect using the Polilight®. Stains on 5 of the 28 fabric types achieved the maximum rating of 2 denoting weak fluorescence. These were the white cotton T-shirt, blue polyester blouse, white jeans, light blue jeans and blue jeans. A total of 15 of 28 items rated zero for fluorescence.

Diluted Stains

Examination with the Polilight® revealed 18 of the 28 diluted stains had no visible fluorescence. Only diluted stains on the white cotton t-shirt, blue fleecy jumper and white jeans achieved a rating of 2 for fluorescence.

Smear Stains

Only 1 smear stain of the 28 achieved a rating of 2 for fluorescence, this being on the blue fleecy jumper. Three of 28 stains were rated 1 for very weak fluorescence, and these were on the white cotton t-shirt, purple pants and white jeans. Smear stains were not detectable with the Polilight® on a total of 24 of the 28 items of clothing. Examples of neat, dilute and smear stains are shown in Figure 3-2.

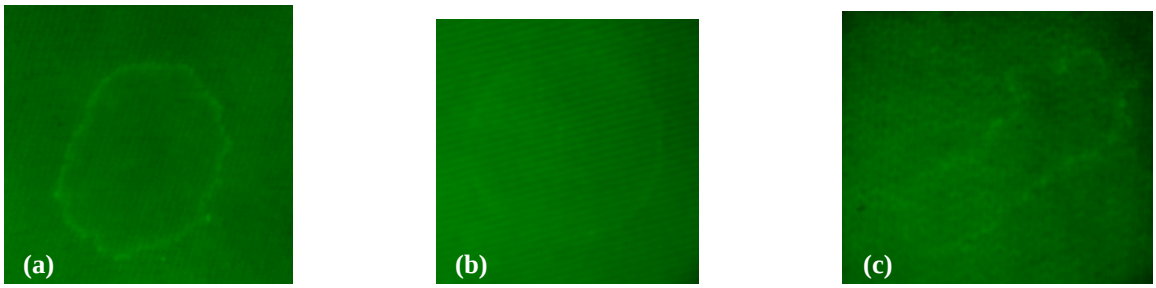

Figure 3-2: Examples of (a) neat saliva stain, (b) 1 in 4 dilute saliva stain and (c) smear saliva stain fluorescence

Residual Staining

Residual saliva staining was visible in natural light as a ‘glue-like’ residue on the neat stains of 15 of the 28 items of clothing, and very faintly visible on a further 7. This same ‘glue-like’ residue was visible on diluted stains on only the red cotton t-shirt, black cotton

singlet, red polyester pants and black polyester skirt. For the smear stains, only the red polyester/cotton shorts and red fleecy jumper showed ‘glue-like’ residue.

Other Observations

Fabric type was found to have no influence on the detection of saliva stains using the Polilight®. Fluorescence of stains varied for each category and no trends relating to fabric type were evident.

Saliva stains were found not to be uniform in shape. On cotton and denim the stains were mostly circular with rounded edges while on polyester/cotton stains were much larger and often had jagged edges. On polyester fabric saliva stains were especially large and in the case of the blue polyester blouse staining spread out of the bounds of the 6 x 6cm grid.

The most versatile combination of excitation wavelength and interference filter for screening saliva stains with the Polilight® was 470nm excitation with the 555nm interference goggles. Other successful combinations were 470nm excitation with the 530nm interference filters as well as 450nm excitation with 555nm or 530nm interference filters. In some cases these latter combinations gave the best contrast. Limited success was experienced when using 415nm excitation in combination with yellow goggles for viewing stains on dark fabrics.

3.1.3 Mock exhibits

Large Stains

See Table 7.2

Of the 12 large saliva stains, the one on the dark blue jeans had no fluorescence visible. The stains on the patterned polyester boxer shorts rated 0.5 for barely visible fluorescence and the worn blue jeans, white fleecy jumper and pattern fleecy jumper all rated 1 for very weak fluorescence. All other stains were visible with the Polilight® and were rated 2 for weak fluorescence.

The red polyester/cotton shorts, pattern fleecy jumper and purple fleecy pants were the only articles of clothing with obvious residual saliva staining visible in natural light. Seven of the 12 items had no 'glue-like' residue visible. Of the 12 large saliva stains, 9 were successfully located by an individual not involved in their application, achieved through a combination of natural light and Polilight® examination.

Gags

See Table 7.3

Of the 6 gags, only the white cotton displayed weak fluorescence in the area of saliva staining. Fluorescence was not visible on any of the other gags. Examination in natural light revealed a dry, glistening, 'glue-like' residue on the acrylic/nylon football socks in the area of the sock that was directly in the mouth. For the white cotton and red satin gags the area of fabric that was directly in the mouth was indicated by deformation of the fabric.

Figure 3-3: Material deformation and soiling visible in a white cotton fabric gag, indicating where the gag had been in the mouth.

3.1.4 Comparison of Saliva and Other Fluid Stains

In natural light the physical characteristics of other fluid stains were generally different to that of saliva. A ring of staining was visible for the apple juice stain, the tea and urine stains were yellow in colour and the 'Spray n' Wipe' stain had a blue colour. When examined with the Polilight®, the apple juice stain was found to be the most fluorescent

and much more intense than the saliva stain. Similarly, the 'Spray n' Wipe' and urine stains displayed more intense fluorescence than the saliva stain, the urine stain having some fluorescence in the body of the stain as well as around the rim. The tea, PBS, amylase and sodium hypochlorite stains all appeared very similar in appearance and had equivalent fluorescence intensity to saliva stains.

Polilight® examination of saliva stains on washed cotton revealed reduced or no fluorescence. Very weak fluorescence was observed on the boiled cotton and no fluorescence was observed on the stain on cotton washed in 'Cold Power' brand washing powder.

3.2 Detection with SALIgAE® Spray

3.2.1 Saliva Dilutions on White Cotton

See Table 7.4.1 and 7.4.2

All pressings of neat saliva stains gave a positive yellow reaction within 30 seconds and those of the 1 in 2 dilutions reacted positively in around one minute, for both formulations of SALIgAE® Spray. Positive colour change for the 1 in 4 dilution filter papers varied from 45 seconds (SALIgAE® Spray Formulation 1) to 8 minutes (SALIgAE® Spray Formulation 2). Two of the 1 in 8 dilution pressings showed positive yellow colour in 5 and 8 minutes respectively while the other two had only faint yellow colour at the 10-minute cut-off. One pressing of the 1 in 16 dilution gave a very faint trace of yellow colour at the 10-minute cut-off time (SALIgAE® Spray Formulation 1), however all others were negative.

3.2.2 Saliva on Different Material Types

See Table 7.5

Neat Stains

Eighteen of the 28 neat saliva stains tested positive with SALIgAE® Spray Formulation 1 and 19 of 28 with Formulation 2. Of the positive results, 9 stains for each formulation of

SALIgAE® Spray had double the weight applied in the pressing step. Stains pressed in this manner accounted for approximately half of the positive results.

1 in 4 Dilution Stains

Seventeen of the 28 dilute stains tested with SALIgAE® Spray Formulation 1 gave a positive yellow colour, with 10 of these having extra weight applied in the pressing step. Fourteen of 28 dilute stains tested with SALIgAE® Spray Formulation 2 were positive and of these, 9 had extra pressing weight applied.

Smear Stains

For the smear stains, 19 of 28 tested positive with SALIgAE® Spray Formulation 1 and 20 of 28 tested positive with Formulation 2. Extra pressing weight was applied in 9 of the 19 positive results for SALIgAE® Spray Formulation 1 and 10 of the 20 positive results for SALIgAE® Spray Formulation 2.

3.2.3 Mock Exhibits

See Table 7.6

All of the fabric gags gave a positive result within one minute using the SALIgAE® Spray. Yellow colour development was observed instantly for the cotton and acrylic/nylon sock gags, both of which were worn in the mouth. Both of the nylon/elastane pantyhose facemasks tested negative using SALIgAE® Spray.

3.3 Spotty Paper Testing

See Table 7.7

Most of the Spotty Paper tests resulted in a positive colour change. The neat stain on white unwashed cotton was positive, the 1 in 2, 1 in 4 and 1 in 8 dilution stains were weakly positive, while the 1 in 16 dilution stain tested negative. Positive results were recorded for neat stains on cotton, polyester and fleecy items; for dilute stains on the polyester/cotton, polyester and fleecy and for smear stains on the cotton, polyester/cotton and polyester items. Weak positive results were recorded for the neat stains on

polyester/cotton and denim, dilute stains on denim and fleecy and smear stains on denim. The only negative (trace) result was recorded for the dilute stain on cotton.

The SALIgAE® Spray and Spotty Paper tests had rather different colour changes to indicate a positive result, as shown in Figure 3-3. For the Spotty Paper test, a vibrant blue colour indicated a positive result, whilst for the SALIgAE® Spray test, a pale yellow colour developed after the application of SALIgAE® reagent. The yellow colour on a white test paper background of the SALIgAE® test was noticeably more difficult to detect than the blue colour of the Spotty paper test.

Figure 3-4: Colour changes indicating a positive result for (a) Spotty Paper test, blue colour, and (b) SALIgAE® Spray test, pale yellow colour

4. DISCUSSION

4.1 Polilight® Detection

The Polilight® is commonly employed for the fluorescent detection of semen stains on articles of clothing and bedding and other suitable materials. It has been proposed that the Polilight® may be suitable for detecting saliva stains. However, to date, their fluorescence has not been documented. In this study, saliva staining on a broad but by no means exhaustive selection of fabrics and colours was investigated in white light and using the full range of Polilight® settings and appropriate filters and goggles. This was followed by an investigation into the ability of the Polilight® to detect saliva staining on simulated casework items like large stains and gags.

When examined under natural light, many of the darker items of clothing displayed areas of 'glue-like' saliva residue on the surface of the fabric. Close examination revealed this residue to be also present on several of the lighter coloured fabrics, however on these items the residue was not as obvious due to the lack of contrast between stain and fabric colour. It was hypothesised that an increase in the volume of saliva applied to material may increase the prominence of this residue, however results from the analysis of larger stains suggest otherwise. Under half of the 12 larger stains showed areas of saliva residue, indicating that most of the saliva volume will soak into the fabric. In some cases the Polilight® was found to enhance the visibility of saliva on the surface of clothing as excitation light was reflected from the residue surface. Only the acrylic/nylon sock gag had saliva residue visible in natural light, as a glistening 'glue-like' stain, however the area of mouth contact could be identified on the other in-mouth gags as a gathering of crumpled material.

Generally the optimum Polilight® conditions for locating saliva stains was determined to be 470nm with the 555nm interference goggles, however 470nm with the 530nm interference filters was also found to be useful. The 415nm excitation light with the 555nm interference filters or the yellow goggles, often suitable conditions for viewing fluorescence on darker colours, did not provide any better contrast than the

470nm/555nm combination. It was found to be similarly difficult to detect stains on purple and pink coloured items. Where saliva staining was detectable with the Polilight®, fluorescence was only visible on the outer rim of the stain and not in the centre. Initially it was proposed that the 0-4 grading system used to rate semen fluorescence also be used to rate saliva fluorescence (Williams, Silenieks et al. 2004), but the very weak nature of saliva stain fluorescence only allowed for a scale of 0-2.

Overall the success rate for locating saliva stains with the Polilight® was very poor. Over half of the 28 items of clothing had no saliva staining visible with the Polilight®, and in many cases the fluorescence was rated as very weak. These results are indicative of the general difficulty experienced in detecting saliva stains using the Polilight®. Denim was found to be the best of the 5 fabric types for locating saliva stains, followed by cotton. White and pale colours or worn denim were best for promoting contrast between saliva stain fluorescence and background colour.

Polyester and polyester/cotton blend fabrics yielded poor results for Polilight® detection. Fabric type did not appear to influence the fluorescent detection of saliva stains however fabric colour had a marked influence with 100µL neat saliva stains being nearly completely undetectable on all dark and red coloured fabrics. This is probably due to the very weak nature of saliva fluorescence and the absorption of excitation light into the dark fabrics. The 415nm excitation light with the 555nm interference filters or the yellow goggles, often suitable conditions for viewing fluorescence on darker colours, did not provide any better contrast than the 470nm/555nm combination. It was found to be similarly difficult to detect saliva stains on purple and pink coloured items. However, when a larger volume of saliva was applied staining became more clearly visible.

Different fabric colours and designs obscured the fluorescence of saliva stains. Increasing the volume of saliva increased the visibility of the stain only in some cases. These results indicate that the visibility of saliva stains on patterned materials depends mostly on the nature and colouring of the pattern.

In general saliva stains on fleecy fabrics did not prove any more difficult to detect than stains on other materials. This contrasts previous studies on semen fluorescence where stains on fleecy fabrics were quite difficult to detect (Kobus, Silenieks et al. 2002). This may be due to the fact that the very weak nature of saliva fluorescence prevented detection of stains on most fabrics, so effects of the fleecy material could not be observed.

Increasing the volume of saliva applied to fabric resulted in an increase in visibility of the fluorescence, as several items that previously had no fluorescence now showed evidence of staining visible with the Polilight®. The larger volume of saliva applied in this case was equivalent to a person 'spitting' and thus could well be encountered in real case scenarios. Interestingly, very little fluorescence was visible on the mock gag exhibits. In the cases where the fabric gag was in the mouth, volunteers found themselves continually salivating resulting in the gags becoming saturated in saliva. The fluorescence visible on the white cotton singlet appeared as a ring around the outer edge of the staining and no fluorescence was visible in the centre. This leads to suspicion that saliva itself is not actually fluorescent and is merely mobilising some weakly fluorescent component through the material, causing the illusion of fluorescence.

The examination of staining generated by other fluids revealed that the fluorescence displayed by saliva is not unique. Several of the fluids investigated showed an almost identical ring of weak fluorescence, none of which would have previously been thought of as fluorescent. A most interesting finding was that saliva applied to washed cotton had weaker intensity fluorescence than stains applied to unwashed cotton. This cotton had been washed only in water. Even more noteworthy was that saliva on cotton that had been washed in 'Cold Power' washing powder had no ring of weak fluorescence at all. This suggests that the product being moved through the cotton by the saliva had been completely removed by the 'Cold Power' wash and partly removed by washing in water only. These results suggest that the fluorescent compound may be water-soluble and that saliva is merely acting as a liquid stain and has no intrinsic fluorescence. This is supported by the absence of fluorescence throughout the central portion of saliva stains.

4.2 Detection with SALIgAE® Spray

The SALIgAE® Spray for the Location of Saliva Stains successfully detected neat saliva stains and stains of 1 in 2 and 1 in 4 dilutions. Dilutions greater than 1 in 4 produced inconsistent results, so to test the sensitivity of detection in relation to fabric types the 1 in 4 dilution was chosen for application to the 28 clothing items, along with neat and smear stains.

In general, very little difference in performance was observed between SALIgAE® Spray Formulation 1 and Formulation 2. The speed of the positive yellow colour development varied across the range of clothing items tested and neither of the SALIgAE® Spray formulations gave consistently faster results. All neat saliva stains on cotton material were detectable with both formulations of the SALIgAE® Spray, while tests conducted on neat stains on the polyester/cotton blend items gave mostly negative results, again for both formulations of SALIgAE® Spray. For the purpose of this discussion then, the results refer only to the overall performance of the SALIgAE® Spray, not that of the individual formulations.

The contact between the test filter paper and the saliva stain was found to be a critical factor. Initial tests used about 5kg of pressing weight and these gave a low number of positive results. When the pressing weight was doubled, the number of positive results increased. This trend was particularly apparent for stains on the thinner polyester/cotton fabrics, smear stains and 1 in 4 dilution stains. These results indicated that good contact between the test filter paper and the stain is essential to maximise the chance of detecting saliva stains on fabrics using the SALIgAE® Spray.

Overall the 1 in 4 dilution saliva stains took longer to give positive reactions when compared with neat saliva stains on the same items of clothing. This was to be expected as there was less salivary amylase within the stain, hence less transferred to the filter paper and less was available to react with the SALIgAE® Spray reagent. For cotton

fabrics, all smear stains were detectable using the SALIgAE® Spray except those on the patterned shirt. Results for stains on 100% polyester fabric, fleecy fabric and denim were varied and no particular trends were evident.

In general saliva stains on fleecy material were most easily detected with SALIgAE® Spray, with 83% giving positive results. In comparison, only 33% of the saliva stains on polyester/cotton material gave positive results with the SALIgAE® Spray. Overall statistics reveal that the SALIgAE® Spray returned positive results for 107 of a total of 168 saliva stains tested (neat, dilute and smear stains), which equates to 64% of stains. Despite the efforts to assess the capacity of SALIgAE® Spray to detect amylase on different fabric types, the vast variation in wear and thickness of items in each category made it impossible to make clear conclusions.

For the mock exhibits, all of the fabric gags recorded a positive result to the SALIgAE® Spray within 1 minute, with 2 of the 3 fabric gags worn inside the mouth reacting instantly. This rapid result was anticipated as these items were saturated in saliva. Following this it can be concluded that SALIgAE® Spray is reliable in detecting the presence of large amounts of saliva. The two nylon/elastane pantyhose legs worn as facemasks however both returned negative results with SALIgAE® Spray. A reason for this result is that these items had little contact with the mouth during the ten-minutes in which they were worn, hence only a very small amount of saliva would have been deposited on the fabric.

Through the course of the experiment it was determined that 8 pumps of the SALIgAE® Spray was required to ensure sufficient SALIgAE® reagent was applied to re-wet a filter paper (of approximate dimensions 6cm by 18cm) and allow the reaction to occur. To prevent the filter paper drying out and the reaction stopping, it was necessary to place the paper in a snap-seal plastic bag for the ten-minute development period immediately following the application of the SALIgAE® Spray.

4.3 SALIgAE® Spray vs. Spotty Paper Testing

Overall the Spotty Paper test was found to produce more positive results for saliva than the SALIgAE® Spray test. While both tests detected most stains on the cotton and fleecy materials, the Spotty Paper test also convincingly detected stains on the polyester and polyester/cotton blend items which were not detected with the SALIgAE® Spray using identical pressing weight. The Spotty Paper test was also able to detect all three saliva stains on denim where the SALIgAE® Spray detected only one. In this case the SALIgAE® Spray test procedure even had double the weight applied in the pressing step, however this still did not make the procedure more effective than the Spotty Paper test.

The reason for the greater efficiency of the Spotty Paper test in saliva detection most likely lies in the test procedure itself. In testing an area for saliva the spotty paper is pressed directly onto the stain, while the SALIgAE® Spray procedure relies on transfer of the saliva to a filter paper before reagent application. Actual contact of the test reagent with a saliva stain would no doubt produce greater efficiency and sensitivity of detection.

Another difference found in the SALIgAE® Spray and Spotty Paper tests was the colour change indicating a positive result. As was shown in Figure 3-3 the Spotty Paper test produces a vibrant blue colour indicating a positive result, a colour much easier to detect than the pale yellow of the SALIgAE® Spray test. In some cases the pale yellow colour change was found to be quite indistinct from the white filter paper background, making it difficult to detect weak positive results. In comparison the more obvious blue colour change of the Spotty Paper reagent made the task of identifying a positive a great deal easier.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

- Fluorescence of saliva stains appears non-specific and similar fluorescence patterns are seen with other liquid stains.
- The Polilight® can be used to screen items to locate possible saliva stains but is less reliable when compared to semen fluorescence screening, as only some saliva stains produce weak, indistinct fluorescence.
- *SALIgAE® Spray for the Location of Saliva Stains at the Crime Scene* can detect saliva stains in the majority of cases where the saliva is not diluted.
- *SALIgAE® Spray for the Location of Saliva Stains at the Crime Scene* test procedure is not as sensitive as the Spotty Paper test procedure, with difference in effectiveness most likely being due to differences in contact with the stain.
- Increasing the weight applied in the pressing step increases the efficiency of the *SALIgAE® Spray for the Location of Saliva Stains at the Crime Scene* test.

5.2 Recommendations

1. Recommended Screening Procedure for Locating Saliva Stains.

- Visual examination using white light for liquid stains, in particular those with a glue-like residue that could indicate saliva staining.
- Polilight® examination primarily using 470nm excitation wavelength/555nm goggle combination, followed by the 470nm excitation wavelength/530nm goggle combination.
- If no stains are detected with the Polilight®, screen the item with the *SALIgAE® Spray for the Location of Saliva Stains at the Crime Scene*, in similar way to Spotty Paper screening.
- If possible saliva staining is identified, the presence of saliva should be confirmed by sampling and testing with the *SALIgAE™ Test for the Forensic Identification of Saliva* test.

2. Recommend The Investigation Into Saliva Fluorescence.

The results of this project indicate that saliva itself does not fluoresce. It appears that saliva acts merely as a fluid stain and further work investigating the fluorescent properties of saliva is recommended to confirm these findings.

3. The Confirmation of Saliva Stains.

The *SALIgAE™ Test for the Forensic Identification of Saliva* SALIgAE® has been shown to be a more sensitive and reliable test compared to the *SALIgAE® Spray for the Location of Saliva Stains at the Crime Scene*. It is recommended that where staining has been located through visual or Polilight screening, that the vial test be used to confirm the presence of saliva.

6. REFERENCES AND ACKNOWLEDGEMENTS

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2. The volunteers for providing the samples used in this project.

7. APPENDIX 1: TABLES

Table 7.1: Results of Polilight® detection of neat, dilute and smear saliva stains on the 28 articles of clothing

Type of Material	Description	Wear (approximate)	Visible with Naked Eye?	Overall Polilight Rating	Stains Visible									Best Filter/ Goggle Combination	Other useful Combinations
					N1	N2	N3	D1	D2	D3	S1	S2	S3		
Cotton	White T-shirt	old	slightly	2	R 2	2	-	2	2	-	1	1	-	470/555nm	-
	Pale blue singlet	medium	no	1	1	1	-	0.5	0.5	-	0	0	-	470/555nm	470/530nm
	Red T-shirt	medium	yes	0	R	R	-	R	0	-	0	0	-	-	-
	Black singlet	medium	yes	0	R	R	-	R	R	-	0	0	-	470/555nm	415nm/yellow goggles
	Patterned shirt	old	no	1	0.5	1	-	0.5	0	-	0	0	-	450/555nm	450nm/orange goggles
Polyester/	White shirt	old	slightly	1	R 2	1	-	1	1	-	0	0	-	470/555nm	470/530nm
Cotton	Pale-blue hooded vest	medium	no	0	0	0	-	0	0	-	0	0	-	470/530nm	-
	Red shorts	medium	yes	0	R	R	-	0	0	-	R	0	-	-	-
	Black shorts	old	yes	0	R	R	-	0	0	-	0	0	-	470/555nm	-
	Check pattern pants	medium	yes	0	0	R	-	0	0	-	0	0	-	-	-
Polyester	White shorts	medium	slightly	0	0	R	-	0	0	-	0	0	-	-	-
	Light blue blouse	medium	very slightly	2	R 2	R 2	2	1	1	1	0	0	0	470/530nm	-
	Red pants	medium	yes	0	R	R	-	0	R	-	0	0	-	-	-
	Sheer black skirt	medium	very clear	0	R	R	-	0	0	-	0	0	-	470/555nm	470/530nm
	Pattern skirt	old	yes	0	0	R	R	R	0	R	0	0	0	450/555nm	450nm/orange goggles
	Pattern boxer shorts	old	no	0.5	0.5	0	-	0	0	-	0	0	-	470/530nm	-
Fleecy	White jumper	medium	slightly	1	1	1	-	1	R 1	-	0	0	-	470/555nm	-
	Blue jumper	old	yes	2	R 1	R 1	-	1	2	-	1	2	-	470/555nm	490/555nm, 505/555nm
	Red jumper	old	yes	0	R	R	-	0	0	-	R	0	-	-	-
	Dark navy pants	medium	yes	0	R	R	-	0	0	-	0	0	-	470/555nm	-
	Pattern jumper	old	yes	0	R	R	-	0	0	-	0	0	-	-	-
	Purple pants	old	very slightly	0.5	0.5	R 0.5	-	0	0	-	0.5	0	-	470/530nm	-
Denim	White jeans	medium	no	2	2	2	-	2	2	-	1	0	-	470/555nm	-
	Light blue jeans (worn)	old	no	2	2	2	-	1	1	-	0	0	-	470/555nm	490/555nm, 505/555nm
	Blue jeans (worn)	old	yes	2	2	2	-	1	1	-	0	0	-	415/555nm	415nm/yellow goggles
	Red jeans	old	yes	0	R	R	-	0	0	-	0	0	-	-	-
	Dark blue jeans (new looking)	medium	yes	0	R	R	-	0	0	-	0	0	-	-	-
	Black skirt	old	slightly	0.5	0.5	0	-	0	0	-	0	0	-	470/555nm	-

***R = Glue-like residue visible**

N = Neat stain

D = Dilute stain

S = Smear stain

Table 7.2: Results of Polilight® detection of larger saliva stains on clothing items

Type of Material	Description	Visible with Naked Eye?	Polilight Rating	Was the stain located?	Location of Stain (coordinates)	Old Best Filter/ Goggle Combination
Cotton	White T-shirt	no	2	located	(4,6)	470/555nm
	Patterned shirt	no	2	located	(3-4,5-6)	450/555nm or 450/orange
Polyester/	Pale-blue hooded vest	slightly	2	located	(5,2)	470/530nm
Cotton	Red shorts	yes	2	located	(1-2,3)	-
Polyester	White shorts	no	2	located	(2-3,2)	-
	Light blue blouse	no	2	located	(3,3-4)	470/530nm
	Pattern boxer shorts	no	0.5	not located	(2,2)	470/530nm
Fleecy	White jumper	no	1	located	(5,5)	470/555nm
	Pattern jumper	yes	1	lots of interference	(2,4), (5,3), (10,2)	-
	Purple pants	yes	2	located	(2,3)	470/530nm
Denim	Blue jeans (worn)	no	1	located	(5,5)	415/555nm or 415/yellow
	Dark blue jeans (new looking)	slightly	0	barely visible	(3,3)	-

Table 7.3: Results of Polilight® detection of fabric mouth gags and face-masks

Type of Material	Description	Position	Visible with the naked eye?	Polilight Rating	Best Filter/ Goggle Combination
Cotton	White Singlet	in-mouth	yes - crumpled where material was in the mouth	2	470/555
Polyester/Cotton	blue check shirt	around mouth	no	0	none
Acrylic/Nylon	blue/yellow/white socks	in-mouth	yes - 'glue-like' stain	0	none
Nylon/Elastane	Black Stockings	around mouth	no	0	none
Unknown	purple satin	around mouth	no	0	none
	red satin	in-mouth	yes - crumpled where material was in the mouth	0	none

Table 7.4: Results of SALIgAE® Spray detection of saliva dilutions on white cotton

Stain	Formulation 1		Formulation 2	
	Run 1	Run 2	Run 1	Run 2
Neat	35secs	30 secs	30 secs	30 secs
1 in 2	1min	45secs	40 secs	1min30secs
1 in 4	2min35secs	45secs	1min40secs	8 mins
1 in 8	5min	very faint at 10 mins	trace only at 10 mins	8 mins
1 in 16	negative after 10 mins	very faint at 10 mins	negative after 10 mins	negative after 10 mins

Table 7.5.1: Results of SALIgAE® Spray Formulations 1 detection of neat, dilute and smear saliva stains on the 28 articles of clothing

Item	Formulation 1							Notes
	pumps	positive development time			after several hours			
		Neat	Dilute	Smear	Neat	Dilute	Smear	
Cotton								
White T-shirt	5	7min	10min	10min	positive	positive	positive	
Pale blue singlet	6	10min	10min	8min30sec	positive	positive	positive	
Red T-shirt	5	1min40sec	10min	2min	positive	positive	positive	weight doubled
Black singlet	8	4min	10min	4min	positive	positive	positive	weight doubled
Patterned shirt	6	10min	negative	negative	positive	positive	positive	
Polyester/Cotton								
White shirt	6	negative	negative	negative	positive	negative	positive	
Pale-blue hooded vest	7	negative	10min	5min	positive	positive	positive	weight doubled; but fabric still appeared dry
Red shorts	7	negative	negative	negative	negative	negative	positive	
Black shorts	8	30sec	5min	10min	positive	positive	positive	weight doubled
Check pattern pants	7	negative	negative	negative	positive	negative	negative	
Polyester								
White shorts	8	10sec	10min	3min	positive	positive	positive	weight doubled
Light blue blouse (stains 1 & 3)	6	negative	negative	negative	positive	positive	positive	
Red pants	5	negative	negative	negative	positive	negative	negative	
Sheer black skirt	5	7min	10min	7min	positive	positive	positive	
Pattern skirt	8	20sec	4min	30sec	positive	positive	positive	weight doubled; stitching of skirt may have caused some troubles for formula 2
Pattern boxer shorts	8	40sec	8min	2min	positive	positive	positive	
Fleecy								
White jumper	5	negative	negative	6min	negative	negative	positive	
Blue jumper	5	1min40sec	10min	1min30sec	positive	positive	positive	
Red jumper	8	7min	2min	1min30sec	positive	positive	positive	weight doubled; bottom one (formula 2) may not have been in contact enough
Dark navy pants	8	1min30sec	4min	1min30sec	positive	positive	positive	weight doubled
Pattern jumper	7	4min30sec	negative	9min	positive	positive	positive	
Purple pants	8	3min30sec	10min	10min	positive	positive	positive	weight doubled
Denim								
White jeans	6	2min20sec	negative	10min	positive	positive	positive	
Light blue jeans (worn)	4	negative	negative	negative	negative	negative	negative	potentially not enough of formula 1 added
Blue jeans (worn)	8	1min30sec	negative	negative	positive	negative	positive	weight doubled
Red jeans	3 per stain	1min30sec	4min30sec	4min	positive	positive	positive	individual hand press for each stain
Dark blue jeans	7	-	8min	8min30sec	positive	positive	positive	
Black skirt	8	negative	8min	negative	positive	positive	positive	weight doubled

Table 7.6: Results of SALIgAE® Spray testing of fabric gags and facemasks

Type of Material	Description	Type of Gag	Positive Development Time		Notes
			Formulation 1	Formulation 2	
Cotton	White Singlet	in-mouth	instant	-	extra weight was not added to the pressing of the mock exhibits
Polyester/Cotton	blue check shirt	around mouth	-	1 minute	
Acrylic/Nylon	blue/yellow/white socks	in-mouth	instant	-	
Nylon/Elastane	Black Stockings	mask over face	negative	negative	
Unknown	purple satin	around mouth	10 seconds	-	
	red satin	in mouth	-	30 seconds	

Table 7.7 (two parts): SALIgAE® Spray testing compared with Spotty Paper testing on the same saliva stains

Item	SALI G						Notes
	Formulation 1 positive development time			Formulation 2 positive development time			
	Neat	Dilute	Smear	Neat	Dilute	Smear	
Dilutions	positive for neat, 1/2, 1/4; slight for 1/8; negative for 1/16			positive for neat, 1/2, 1/4, 1/8; negative for 1/16			
Black Cotton	4min	10min	4min	4min	10min	2min	weight doubled
Pattern Poly/Cotton	negative	negative	negative	negative	negative	negative	
Red Polyester	negative	negative	negative	negative	negative	negative	
Dark Fleecy	1min30sec	4min	1min30sec	3min	6min	3min	weight doubled
Black denim	negative	8min	negative	negative	negative	negative	weight doubled

Item	Spotty Paper		
	Neat	Dilute	Smear
Dilutions	positive for neat, weak for 1/2, 1/4, 1/8, negative for 1/16 (trace)		
Black Cotton	positive	negative (trace)	positive
Pattern Poly/Cotton	positive (weak)	positive	positive
Red Polyester	positive	positive	positive
Dark Fleecy	positive	positive	positive (weak)
Black denim	positive (weak)	positive (weak)	positive (weak)

8. APPENDIX 2: METHOD PROTOCOLS

SPOTTY PAPER TEST PROCEDURE

SalivaSpot(1.0) Commencement Date: 25 September 2001

1. Scope

This procedure describes the presumptive testing for the presence of saliva stains and can be applied to a wide range of textiles including clothing and bedding.

2. Principle

Saliva stains can rarely be located by observation and their detection is dependent on chemical testing.

The Spotty Paper test relies on the presence of high concentrations of a-amylase in saliva. The test paper is impregnated with a water-insoluble cross-linked starch polymer carrying a blue dye. In the presence of a-amylase, the starch is hydrolysed to form water-soluble blue fragments.

3. Validation

Willcott GM and Griffith M. A new method for locating saliva stains – Spotty Paper for spotting spit. Forensic Science International 15 1980 79-83.

4. Reagents

4.1 distilled water

4.2 Phadebas Amylase Test tablets

4.3 Preparation of Spotty Paper

4.3.1 Triturate one Phadebas tablet with a mortar and pestle.

4.3.2 Transfer to a Universal bottle and suspend in 10mL of distilled water

4.3.3 Suspend a 20 x 20cm Whatman No 1 filter paper in the fume hood and spray the suspension onto the paper using an aerosol sprayer.

4.3.4 Allow to dry.

4.3.5 Mark the treated surface of the paper.

4.3.6 Place the dried paper into a plastic bag and label with initials, date and expiry date (6 months from the date of preparation).

5. Apparatus

5.1 Whatman No 1 filter papers, 20 x 20 cm

5.2 aerosol sprayer

5.3 Universal bottle

5.4 misting sprayer

5.5 glass plates

5.6 weights

5.7 incubator 37ºC

5.8 plastic sheet

5.9 examination board

6. Procedure

6.1 Wash the examination board thoroughly with detergent.

6.2 Pre-warm the examination board, weights and glass plate in the incubator at 37ºC

6.3 Lay the item on the examination board. In the case of double layered garments or bedding, place another clean examination board between the layers to prevent cross-contamination.

6.4 Take care to avoid touching the treated surface of the Spotty Paper.

6.5 Lightly dampen the treated surface of the Spotty Paper with distilled water using a misting sprayer.

6.6 Place the treated surface of the Spotty Paper onto the item and mark the location on the item with a chinagraph pencil.

6.7 Place a plastic sheet over the Spotty Paper followed by a glass plate and the weights to ensure good contact between the item and the spotty paper.

6.8 Incubate the item / Spotty Paper at 37ºC for 45 minutes.

6.9 Record the presence of any pale blue zones against the mottled blue background. These zones may be more clearly visible when the paper has dried.

6.10 Run a saliva positive control stain and record its use, expiry date and result on the worksheet.

6.11 If a further area of the item is to be tested for saliva, clean the examination board with alcohol and repeat the test.

7. Interpretation of Results

7.1 The development of pale blue zones against the mottled blue background within 45 minutes indicates a positive test result for a-amylase.

7.2 A positive test result is taken as presumptive evidence for the presence of saliva.

7.3 Low levels of amylase activity may be found in semen, vaginal and urine stains and higher levels in faecal stains. Thus, the presence of amylase activity alone is not necessarily a conclusive test for saliva.

7.4 Some individuals produce saliva with little or no amylase activity and, therefore, a negative test result does not necessarily exclude the presence of saliva (see Notes.)

Notes:

· A useful further test for the presence of saliva is to prepare a smear from the suspect area, stain with Christmas Tree stain and examine under the high power microscope for the presence of buccal epithelial cells.

· The level of amylase activity in blood and semen is sufficiently low for saliva to be detected in admixture with both.

ABACUS SALIGAE® SPRAY TEST PROCEDURE