FORENSIC BULLETIN: TECHNICAL NOTE

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The Detection of Salivary Amylase in Expirated Blood Patterns

When interpreting bloodstain patterns at crime scenes, identifying the presence of saliva in possible expirated blood may assist in determining whether a pattern has been caused by the blood being expirated or from impact spatter. Expirated blood patterns are typically formed by a person suffering from a serious head wound or internal injury, coughing or sneezing blood droplets onto nearby surfaces[1]. The resultant spray pattern can consist of distinct small stains or spots. A similar pattern of small blood droplet stains can be formed on nearby surfaces when a blunt force such as a weapon impacts a bloodied surface resulting in impact spatter. Distinguishing between the two patterns can often be difficult, with interpretations relying on physical characteristics and case scenario information. Typically, small expirated blood droplets may contain air bubbles or may have a diluted appearance due to other body fluids, such as saliva, being mixed with the blood as it is coughed from the mouth.

A new test for salivary amylase has been developed by Abacus Diagnostics, the *SALIgAE® Test for the Forensic Identification of Saliva*[2] *(or the "Sally-G Test").* This test has been used to identify the presence of salivary amylase in samples taken from expirated bloodstains at a Blood Pattern Analysis training workshop recently held in Adelaide. The test provided rapid results and is suitable for use in forensic laboratories and at crime scenes.

Materials and Methods

Approximately 30mls of blood was drawn from two volunteers who immediately transferred their own blood into their mouths, drew it back into their throats and coughed

the blood onto walls covered with white paper and onto their protective coveralls. Three types of patterns were produced as follows:

Pattern One: This pattern consisted mainly of moderate to large splashes of blood projected onto the target surface, which did not appear to be diluted by saliva. Smaller bloodstains which also appeared undiluted, surrounded the heavy blood splashes. The pattern simulated large volumes of blood that can be coughed up if a person is suffering from internal bleeding caused by, for instance, a punctured lung. One sample was taken from a heavy stain using a small Copan Urethral Swab. These swabs are smaller than the typical sterile cotton swabs commonly used for taking samples for forensic purposes.

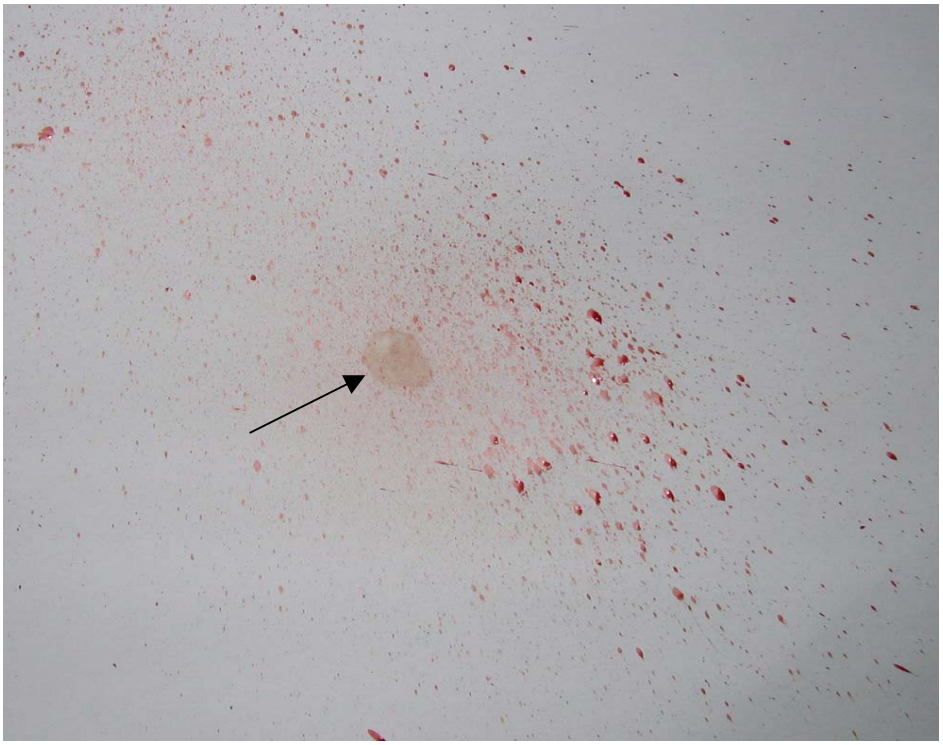
Pattern Two: This pattern consisted mainly of a large splash of blood on the protective tyvek coveralls worn by the volunteer. One sample was taken from this heavy staining using a small Copan Urethral Swab.

Pattern Three: This pattern consisted mostly of small blood droplets that were pale in colour and had an appearance consistent with that of diluted blood. The pattern simulated typical spray patterns associated with expirated blood from the mouth. Two samples were taken from the diluted blood staining using the small Copan Urethral Swabs.

All patterns were allowed to dry for approximately 15 minutes before the swab samples were taken. The swabs from both patterns were dried and tested after approximately 28 hours with the *SALIgAE® Test.*

Pattern 1. The arrow indicates a stain that is approximately 3cm x 1cm. The sample was taken from the top portion of the stain.

Pattern 2. The arrow indicates the area of heavy blood splash sampled from the coveralls. Sufficient sample was taken to darken the swab.



Pattern 3. The arrow indicates the area of fine staining where both samples were taken from This was approximately 2cm in diameter.

The *SALIgAE® Test*

The *SALIgAE® Test* relies on the amylase present in saliva reacting with a colourless test solution to produce a strong yellow colour change that can be observed in as little as one minute, with a maximum time limit of ten minutes for the test. A negative result is indicated by the lack of a colour change within the 10 minute time frame. As little as $2mm^2$ of a suspected saliva stain must first be extracted with either distilled water or phosphate buffered saline. 8ul of sample extract is then added to the test solution, which is contained in a small test vial. In the case of bloodstains, extracts may be discoloured by the presence of heme in the solution and may have to be diluted prior to the addition of the 8ul to the actual test solution. This prevents excessive discolouration of the test vial solution, which can mask the positive yellow colour change. Validation work at Forensic Science SA has shown that for strong bloodstain extracts, a maximum dilution of 1 in 20 is recommended.

One half of each swab sample taken from the mock expirated bloodstains was extracted with 20ul of phosphate buffered saline for 30 minutes. Sample extracts from the heavy blood splash samples and from the weaker typical expirated blood patterns were tested undiluted. The heme present in the heavy blood splash extracts discoloured the test vial solution, so 1 in 20 dilutions were also tested. 8ul of each was added to a test vial and results recorded.

Results

The undiluted samples from the heavy splashes on the wall and coveralls produced weak positive results *SALIgAE®* test results at ten minute time limit, indicating the presence of salivary amylase in both samples. The 1 in 20 dilutions were negative at ten minute time limit, however a trace amount of colour was observed in the 1 in 20 sample taken from the wall. This colour was not strong enough to indicate a positive reaction for salivary amylase. Both samples taken from pattern three gave positive test reactions after two minutes and were strongly positive at the ten minute time limit for the *SALIgAE®* test, indicating the presence of salivary amylase in both samples.

Discussion

The *SALIgAE® Test for the Forensic Identification of Saliva* has undergone an extensive validation study at Forensic Science, South Australia. As part of this validation, 10 mixed blood/saliva stains were prepared on washed cotton cloth by depositing 50µL of saliva onto a $10mm^2$ bloodstain. The mixed stains were dried for one hour and then tested with the *SALIgAE®* test. The 10 mixed stains gave strong positive results to the *SALIgAE®* test while the 10 neat blood samples all tested negative.

Although limited expirated samples were tested in this study, results indicate that *SALIgAE® Test for the Forensic Identification of Saliva* may be a useful tool in assisting the Blood Pattern Analyst in distinguishing between expirated blood and impact blood spatter patterns. Positive *SALIgAE®* results, combined with the physical characteristics of the stains within the pattern, could give the blood pattern analyst a rapid indication that the stain contains saliva and has therefore been expirated from the mouth. For heavy

bloodstain extracts that require dilution, a negative *SALIgAE®* result should be considered inconclusive as the amount of salivary amylase present in these extracts may be below the sensitivity of the test.

Conclusion

The *SALIgAE® Test* is simple to use, provides rapid results and could be used effectively at crime scenes to detect the presence of salivary amylase in bloodstains. Initial results in this very limited study indicate it has potential to detect salivary amylase in expirated bloodstains located at crime scenes and on items submitted to forensic laboratories for examination. Further validation studies will be undertaken to confirm the suitability of the *SALIgAE® Test* for identification of salivary amylase in stains thought to have been caused by expiration of blood.

References

1. Bloodstain Pattern Analysis, Second Edition. Tom Bevel and Ross M. Gardiner, CRC Press, 2002. Pp 85-87, 219-220.
2. *SALIgAE® Test for the Forensic Identification of Saliva.* Technical Information Sheet. Abacus Diagnostics PTY. LTD.