Quantitative and Qualitative Analysis of Minute Levels of Saliva in Expirated Blood Stains

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Abstract

AIM: The aim of this research is to investigate the ability of SALIgAE® to accurately detect the presence of, and quantity of, trace amounts of saliva within expirated bloodstain patterns. METHODS: Following IRB approval and informed consent, venous blood and saliva was collected from the volunteer. The sensitivity of the SALIgAE® solution was first tested with dilutions of saliva:ddH_20, and saliva:venous blood, ranging from 1:1 to 1:1,000,000. Four individual expirated stains were then created by the volunteer coughing blood onto a surface. 42 resulting bloodspots from each stain were chosen for analysis using the SALIgAE® solution, via visual color change and a spectrophotometric reading. RESULTS: The sensitivity of SALIgAE® with dilutions of saliva:ddH_20,and saliva:venous blood produced positive color changes up to 1:1,000 for both. Of the 42 individual spots tested from each expirated stain, between 8 and 23 spots produced a positive color change, with salivary amylase concentrations ranging 0.00µg/mL to 0.28µg/mL. The results of this study highlight the ability of SALIgAE® to detect the presence of minute quantities of saliva mixed with blood.

Introduction

In forensic investigations, blood evidence is common in cases of violent crime. A major challenge that comes with Bloodstain Pattern Analysis (BPA) is the differentiation of expirated and impact blood spatter stains.

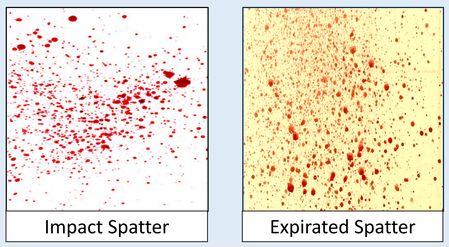

Figure 1. Visual similarities of impact and expirated blood spatter.

Currently, the only accepted method of classifying an expirated bloodstain pattern is the presence of air bubbles in the stain. This is a very subjective approach however, and leaves the assessment open to much scrutiny. As expirated blood is expelled from the mouth, it is logical to assume there would be trace amounts of saliva mixed with the resultant blood droplets. To date however, a method has not yet been identified which is adequately sensitive or specific enough to detect minute traces of saliva in expirated bloodstains.

Objectives

The objectives of this research was to investigate the ability of SALIgAE® to accurately detect the presence of, and quantity of, trace amounts of saliva within expirated bloodstain patterns.

Materials and Methods

Following Institutional Review Board (IRB) approval, and informed consent, venous blood and saliva was collected from the volunteer. The sensitivity of the SALIgAE® solution was first tested with dilutions of saliva:ddH_20, and saliva:venous blood, ranging from 1:1 to 1:1,000,000. Expirated bloodstains were created by placing 1mL of blood into the volunteer's mouth for 30 seconds, followed by the volunteer coughing the blood onto white butcher paper placed approximately 12 inches in front (vertical) and below the volunteers mouth (horizontal).

Figure 2. Collection and creation of samples for testing.

Four expirated stains were created on separate days and 42 individual blood spots/stains were chosen to be tested from both the vertical and horizontal planes of each stain. Each sample was tested with the SALIgAE® solution. Both a visual color change test and a spectrophotometric reading using the Nanodrop™ OneC UV-Vis Spectrophotometer were used to determine the color change and absorption of salivary amylase.

Results

The sensitivity of SALIgAE® with dilutions of saliva:ddH_20 produced the required positive color change up to 1:1,000, as previously reported, with absorbance values ranging from 1.28 to 10.0, and salivary amylase concentrations ranging from 0.12µg/mL to 1.33µg/mL. The sensitivity with dilutions of saliva:venous blood produced the same required positive color change up to 1:1,000, however, the red color of the blood made a distinct color change difficult to observe.

Saliva:Blood Dilutions	Visible Color Change	Concentration of Salivary Amylase (µg/mL)
1:1	✓	2.03
1:10	✓	1.78
1:100	✓	1.72
1:1,000	✓	1.20
1:10,000	✗	0.27
1:100,000	✗	0.19
1:1,000,000	✗	0.24

Table 1. SALIgAE® results, visual and spectrophotometric, of saliva:blood dilutions.

Results Continued

Of the 42 stains selected from each of the 4 expirated stains, between 8-23 produced a positive color change, with absorbance values ranging from 0.08 to 1.05, and salivary amylase concentrations ranging 0.00µg/mL to 0.28µg/mL. While the concentrations obtain from the expirated stains are low, a visible color change did occur.

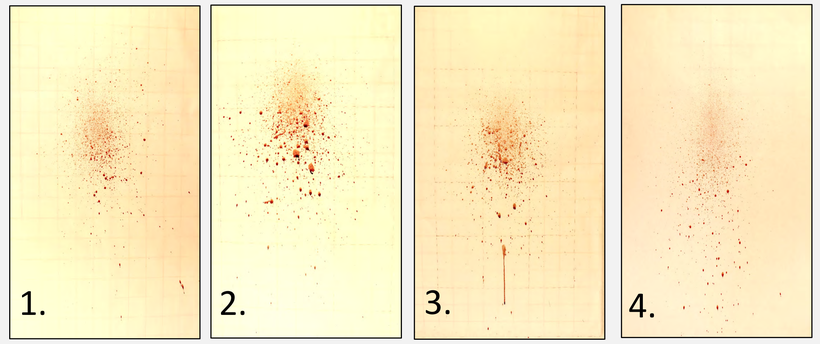

Figure 3. Vertical plane of expirated stains created 1-4.

	Stain #1	Stain #2	Stain #3	Stain #4
# Stains Tested	42	42	42	42
Positive Color Change	8	9	23	10
Salivary Amylase Concentration Range (µg/mL)	0.00 - 0.09	0.00 - 0.15	0.00 – 0.28	0.00 – 0.06

Table 1. SALIgAE® results, visual and spectrophotometric, of expirated stains.

Conclusion

The results of this study highlight the ability of SALIgAE® to detect the presence of minute quantities of saliva when mixed with blood. This reveals the SALIgAE® method to be an ideal candidate for the differentiation of expirated spatter and impact spatter, thereby overcoming a significant challenge facing bloodstain pattern analysts.

This information will ultimately help guide forensic professionals to develop more effective strategies in their processing and analysis techniques.

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