## B3 Qualitative and Quantitative Analysis of Minute Levels of Saliva in Expirated Blood

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The goal of this presentation is to inform attendees of the use of the solution SALIgAE®, which has quantitative and qualitative capabilities to detect minute levels of saliva in expirated bloodstains.

This presentation will impact the forensic science community by providing insight into the difficulties of distinguishing between expirated and impact spatter bloodstains and by informing attendees how the use of SALIgAE® can help minimize those difficulties.

A major challenge with Bloodstain Pattern Analysis (BPA) is the differentiation of expirated and impact blood spatter stains. Currently, the only accepted method of classifying an expirated bloodstain pattern is the presence of air bubbles in the stain; however, this is a very subjective approach 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, a method has not yet been identified that is adequately sensitive or specific enough to detect these minute traces of saliva in expirated bloodstains. SALIgAE® is a somewhat new reagent for saliva identification. This is a clear solution that, when mixed with saliva, turns a transparent yellow color, displaying a positive reaction for saliva. It is reportedly more accurate than other saliva detection tests, with a sensitivity of 1:1,000. Additionally, it has both qualitative and quantitative analyses that are rapid and easy to perform. The goal of this research, therefore, is to investigate the ability of SALIgAE® to accurately detect the presence of, and quantity of, trace amounts of saliva within expirated bloodstain patterns.

Following Institutional Review Board (IRB) approval and informed consent, venous blood was collected from a volunteer into sterile EDTA Vacutainer® tubes. Saliva was also collected from the volunteer into a sterile Falcon® tube. The sensitivity of the SALIgAE® solution was first tested with dilutions of saliva:dd$H_20$ 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 volunteer's mouth (horizontal). Two stains were created on separate days when the volunteer had not drunk liquids or consumed any food for at least one hour. Individual blood spots/stains were chosen to be tested from both the vertical and horizontal planes. Each sample to be tested was incubated in dd$H_20$ for 30 minutes before addition to the SALIgAE® solution. Both a visual color change test and a spectrophotometric reading using the Nanodrop™ OneC Ultraviolet/Visible (UV-Vis) spectrophotometer were used to determine the color change and absorption of salivary amylase. The visual test exhibited a color change from clear to yellow with the presence of saliva, while the UV-Vis test exhibited a change in the absorbance of the saliva and SALIgAE® solution, allowing for the acquisition of quantitative data.

The sensitivity of SALIgAE® with dilutions of saliva:dd$H_20$ 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. The first expirated stain created a large dispersed pattern on the vertical plane, with less abundant but larger drops on the horizontal plane. Forty-two individual stains were selected for testing, each ranging between 1mm and 5mm in diameter. Of the 42 stains, 8 produced a positive color change, with absorbance values ranging from 0.08 to 1.05 and salivary amylase concentrations ranging 0.00µg/mL to 0.09µg/mL. The second expirated stain created a visually similar pattern to the first, and 42 stains were again selected for testing. Of the 42 stains, 9 produced a positive color change, with absorbance values ranging from 0.05 to 1.46 and salivary amylase concentrations ranging from 0.00µg/mL to 0.15µg/mL. While the concentrations obtained from the expirated stains were low, a visible color change did occur.

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.

**Saliva, Expirated Blood, SALIgAE®**



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