

Validation of Abacus SALIgAE® Test for the Forensic Identification of Saliva

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Abstract

Saliva stains can play an important part in the forensic investigation of violent crimes by linking a suspect to DNA from saliva found at a crime scene. The SALIgAE® saliva test from Abacus Diagnostics, a colorimetric test, provides a new alternative to the saliva tests of the past. This test is based upon the presence of salivary amylase, a compound present in high concentrations in human saliva. In this study, samples extracted from mock casework samples as well as dilution series were dispensed into the SALIgAE® test vials and measured for color change against a negative control. All samples as well as the dilutions up to 1:1000 tested positive for saliva with varying degree of color change depending on dilution of the sample. In the sensitivity portion of the study it was found that dilutions up to 1:1000 gave results, but dilutions past 1:400 were difficult to read. All of the samples we expected to be positive gave positive test results, except some samples in the blind soda can study and the ski mask study. The success of this test in the mock casework showed that this test has the potential to give positive results on evidence, such as cigarette butts and soda cans, that we regularly receive in our lab. Based on the results from this study the SALIgAE® test has proven to be sensitive, simple, accurate, and could become a valuable tool to our lab.

Introduction

Saliva stains can play an important part in the forensic investigation of violent crimes. Once detected, DNA from a saliva stain can be traced back to an individual and provide valuable evidence in their prosecution. Saliva stains can often be found on the licked portion of bomb threat letters or on the perimeter of a bite mark. Because saliva is encountered and collected much less frequently than blood or semen in evidentiary materials, it has received less attention and research; therefore many crime labs rarely use saliva tests except in areas where saliva is sure to be found. A new test to better identify this potentially important type of evidence would provide the forensic investigator with a valuable tool for finding saliva stain DNA evidence.

Amylase is a compound found in the body fluids of humans, as well as all primates and most mammals (Willott, 1974). Pancreatic and salivary are the two forms of amylase found in human body fluids. These structurally different amylases are encoded by separated loci on chromosome 1 (Merrit et. al., 1973). Although the distribution of pancreatic and salivary amylase can vary in body fluids, it has been reported that salivary amylase is found in saliva, perspiration, and breast milk (Merrit et. al., 1973). Pancreatic amylase is found in semen, feces, and vaginal secretions (Merrit et. al., 1973). Salivary amylase is the particular form that is focused on for the forensic identification of saliva. Salivary amylase is produced in the salivary glands and begins starch digestion in the mouth. Most of the old methods for the forensic detection of saliva cannot distinguish between salivary and pancreatic amylase.

The presumptive identification of saliva has conventionally been performed by the detection of amylase using techniques such as the Phadebas® assay (Willott, 1974) or radial diffusion in a starch/agarose gel (Schill, Schumaker, 1972). The Phadebas assay works by releasing a blue color when amylase activity breaks open a blue starch substrate (Willot, 1974).

These amylase tests cannot make a distinction between the amylases commonly found in microorganisms, plants, and many animals from those found in humans. Some other drawbacks to these methods are long assay times, and specific instrumentation requirements. They also many suffer from endogenous glucose interference, give unstable reaction colors resulting in poor reproducibility, and the previously mentioned lack of specificity (Quarino et. al., 2005). In starch-iodine tests, only a specific part of the substrate is measured and the enzyme does not work under conditions of substrate saturation. A high rate of false positives and false negatives make many of these tests unreliable in forensic casework. Other attempts to develop more specific methods have been made, such as using selective anti-sera (Eckerson et. al., 1981), chemical inhibitors (Quarino et. al., 1993), and inhibiting monoclonal antibodies (Gerber et. al., 1987).

The SALIgAE® saliva test from Abacus Diagnostics provides a new alternative to the amylase tests of the past. It is a solution in a tube that changes color with the addition of an extract containing saliva. The exact mechanism of the SALIgAE® test is not known due to its proprietary nature and commercially unreleased status. If validation shows this test to be accurate in the identification of saliva, it could provide any forensic lab with a valuable tool for locating and identifying this valuable type of evidence.

Methods

Saliva samples for this study were collected from various staff of the WVSP crime lab. In most of the extraction runs, the saliva was recently obtained from a donor, but in the fourth extraction run the saliva used for the run was collected a month before the test was done. Samples were collected into 50 ml plastic conical tubes and stored in a refrigerator until use.

Samples were vortexed for five seconds immediately prior to use. In experimentation, recently calibrated micropipettes and autoclaved microtubes were used to ensure accuracy and prevent contamination.

In the extraction portion of the study, 50 μ l of whole saliva was dispensed onto a circled area of a filter paper. This filter paper was then dried in a drying chamber for 30 minutes. After the filter papers were dried, 1/8in. x 1/8in cuttings were taken from the saliva stained areas. These cuttings were placed 1.5 ml microtubes, labeled 1S-3S and 1E-4E. The samples were incubated in either 100 μ l or 200 μ l of distilled water. The samples were then incubated for 30 minutes at room temperature on a Nutator in order to extract the saliva stain from the substrate. The samples were then vortexed and 8 μ l of the extract was aliquoted into the SALIgAE® test solution. The contents of the test vial were mixed and it was noted when color change first occurred and the degree of color change at the end of the ten minute test period. The color change was compared against a negative control. The negative control was an unused SALIgAE® test vial with solution.

In the sensitivity component of the study, two separate dilution series tests were performed. In the first dilution series, 50 μ l of saliva diluted by distilled water 1:1, 1:2, 1:4, 1:10, 1:20, 1:100, 1:1,000, 1:10,000, and 1:100,000 was dispensed onto filter paper. In the second dilution series, only 1:1, 1:10, 1:20, 1:100, 1:400, 1:1000, 1:10,000, and 1:100,000 were used. This dilution series followed somewhat the dilutions used in the WVSP validation of the starch/amylase test. The series was diluted in this manner: 1:1 (20 μ l saliva), 1:2 (10 μ l 1:1, 10 μ l H₂O), 1:4 (10 μ l 1:2, 10 μ l H₂O), 1:10 (10 μ l 1:4, 15 μ l H₂O), 1:20 (10 μ l 1:10, 10 μ l H₂O), 1:100 (10 μ l 1:20, 40 μ l H₂O), 1:1000 (10 μ l 1:100, 90 μ l H₂O), 1:10,000 (10 μ l 1:1000, 90 μ l H₂O), 1:100,000 (10 μ l 1:10,000, 90 μ l H₂O). Distilled, PCR-Grade H₂O was used in all of the

dilutions and throughout the experiment. After preparation, 8 μ l of each dilution was added to a labeled SALIgAE® test. Color changes were noted and results were called after the ten minute test period. The color change was compared against a negative control.

In the next part of the study, envelopes were used to simulate cases where saliva from an envelope seal may make the link between a suspect and a crime. Three types of envelopes were used: white business envelopes “W”, pink business envelopes “P”, and manila storage envelopes “M”. These envelopes were sealed by staff at the WVSP by licking the seal area. After sealing, the envelopes were put into the drying chamber for one hour. Each envelope was then taken out of the drying chamber and cut in half perpendicular to the seals. One half of each envelope was used for the opened portion of the test and one half for the unopened portion. This was done because envelopes are brought into crime labs in both the opened and unopened state. In the opened test “O”, seals were pulled apart and 1/8 in x 1/8 in cuttings were taken where the envelope appears to have been sealed. In the unopened test “U”, seals were left intact and 1/8 in x 1/8 in cuttings were taken where the envelope appears to have been sealed. A 1/8 in x 1/8 cutting was taken from the seal area of a pink envelope that was sealed with only H₂O in order to make sure the glue was not causing the positive reading. Cuttings were placed in labeled tubes with 50 μ l of distilled water and extracted on a Nutator for 30 minutes. 8 μ l of the extract was dispensed into SALIgAE® test vials. The “MU” extract was diluted by 50 μ l more of H₂O to dilute the yellow color from the manila envelope in order to prevent a false positive. Color changes were noted and results were called after the ten minute test period. The color change was compared against a negative control.

In the next case study, plastic soda bottle and aluminum soda cans were used to simulate cases where saliva from the rim of a bottle/can may link a suspect to the scene of a crime. A

plastic bottle and an aluminum can were cleaned and wet with saliva along their rims by wiping them with a saliva soaked swabs. The bottle and can were then placed in the drying chamber for two hours. Swabs, moist with H₂O, were wiped along the inside and outside rims of the bottle and can to collect the saliva stains. These swabs were then dried in the drying chamber for eight hours. Small cuttings were made from the swabs, cuttings were placed in a tube with 200 µl of distilled H₂O, and the cuttings were incubated on a Nutator for 30 minutes in order to extract the saliva. 8 µl of the extract was dispensed into SALIgAE® test vials. Color changes were noted and results were called after the ten minute test period. The color change was compared against a negative control.

In the specificity part of the study swabs were soaked in the following samples collected by WVSP staff: cat saliva, dog saliva, human saliva, semen, vaginal secretions, sweat, and blood. Small cuttings from swabs extracted in 200 µl H₂O for 30 minutes at room temperature. An extra 200 µl of H₂O added to blood the extract to dilute the color. 8 µl of each extract dispensed into labeled test vials. Color changes were noted and results were called after the ten minute test period.

In the next case study, cuttings were taken from the mouth area of two ski masks used in a Breaking & Entering. The cuttings were extracted in 200 µl H₂O for thirty minutes at room temperature. 8 µl extract dispensed into labeled test vials. Color changes were noted and results were called after the ten minute test period.

In the soda can blind study, four cans were collected from a recycling bin. One can was washed, and then wiped with a saliva soaked swab as a positive control. Each can was swabbed with H₂O moist cotton swabs. The swabs were air dried for 12 hours in a drying chamber. Small cuttings were then taken from each swab and extracted in 200 µl H₂O for 30 minutes at

room temperature. Eight μ l extract dispensed into labeled test vials. Color changes were noted and results were called after the ten minute test period.

In the cigarette filter blind study, seven cigarette filters were collected from ash trays (some in the weather and some out of the weather). One unsmoked cigarette collected as a negative control. After the cutting was taken of the negative filter, 100 μ l of saliva was applied to the filter and allowed to dry overnight as a positive control.

Results

In the first extraction run (Figure 1), both “1S” and “2S” showed a faint yellow color compared to the negative control. “1S” and “2S” both tested positive for saliva.

In the second extraction run (Figure 2), “3S” showed a yellow color change after only 2 minutes. The color change was intensified by ten minutes. “3S” tested positive for saliva.

In the third extraction run (Figure 3) “1E” showed a pale yellow color change almost immediately, and a more intense color change after four minutes. “2E” showed a faint yellow color change after four minutes and was a pale yellow color at ten minutes. Both “1E” and “2E” tested positive for saliva.

In the fourth extraction run (Figure 4) “E3” showed a pale yellow color change almost immediately and a more intense color change after eight minutes. E4 showed a pale yellow color change almost immediately and a more intense color change after ten minutes. Both E3 and E4 tested positive for saliva.

In the first whole saliva dilution series (Figure 5) 1:1, 1:2, 1:4, and 1:10 showed an intense yellow color change almost immediately. The 1:20 dilution showed a yellow color change after one minute and was more intense after ten minutes. The 1:100 dilution and 1:1000

showed a pale yellow color change after about six minutes. Each dilution from 1:1-1:1000 tested positive for saliva.

In the second whole saliva dilution series (Figure 6) 1:1, 1:10, and 1:20 showed a strong yellow color change almost immediately. The 1:100 dilution showed a pale yellow color change almost immediately and showed a strong yellow color change by ten minutes. The 1:400 dilution showed a faint yellow color change after four minutes. The 1:1000 dilution showed a very faint color change at ten minutes and there was no visible color change in 1:10,000 or 1:100,000. All dilutions from 1:1-1:1000 tested positive for saliva.

In the envelope mock casework study (Figure 7) “PU” and “PO” both showed a pale yellow color change after five minutes, strengthening somewhat by ten minutes. “PO” showed the stronger of the two color changes. “P-“ showed no color change after the ten minute test period. “WU” and “WO” showed a pale yellow color change at eight minutes, with “WO” showing the stronger color change. The extract from “MU” and “MO” was already a pale yellow, but both “MU” and “MO” showed an intensified yellow color by six minutes, with “MU” having the stronger yellow color.

In the bottle/can swabbing mock casework (Figure 8) both “B1” and “C1” showed a pale yellow color change almost immediately and an intense color change after two minutes. Both “B1” and “C1” tested positive for saliva.

In the specificity study (Figure 9) the human saliva showed a strong color change after two minutes. None of the other samples tested showed a visible color change. The cat saliva, dog saliva, human semen, vaginal secretions, sweat, and blood all tested negative.

In the ski mask case study (Figure 10, Figure 11) ski mask SM1 showed a strong color change after only 3 minutes. Ski mask SM2 showed no visible color change. Only SM1 tested positive for saliva.

In the soda can blind study (Figure 12) cans 3 and 5 showed a pale yellow color change after 2 minutes that was stronger at ten minutes. Cans 1,2, and 4 showed no visible color change. In the second soda can blind study can 7 showed a pale yellow color change after ten minutes. Cans 6 and 8 showed no visible color change. Only cans 3,5, and 7 tested positive for saliva.

In the cigarette filter blind study (Figure 13) all samples showed at least a pale yellow color change at ten minutes, with C3, C4, and C6 showing the strongest. All cigarette filters, except the negative control, tested positive for saliva.

Discussion

From the extraction tests in the study, it was determined that 100 μ l is the optimal amount of H₂O for extracting saliva samples from cuttings. A volume of 200 μ l gives good results also and could be a better volume if it was found that a contamination of other body fluids can cause a false positive. In the study, it was found that the amount of color change depends on the dilution of the sample, so 200 μ l may be too much of a dilution for some casework samples with very small saliva stains. With the success of the extractions from the extraction runs and from the casework studies, it appears that 1/8 in. X 1/8 in. is a suitable sized cutting for extracting saliva. Also, in extraction run four, it was found that a saliva sample still gives positive results a month after being collected. This means that evidence that is not tested immediately will probably still be able to give positive results. Any differences in intensity of the color change

between samples in the same parts of the study are probably a result of getting a cutting where more saliva was deposited than another.

In the sensitivity portion of the study it was found that dilutions up to 1:1000 can still give results, but that they are hard to distinguish past 1:400. Results from Abacus indicated that the limit of detection is around 1:2500 and the limit of good intensity color change is 1:500, so our results are comparable to theirs. The intensity of the color changes in the casework study falls in between the 1:100 and 1:1000 dilution.

The success of the test in the envelope study and cigarette filter blind study showed that this test can give positive results on the kinds of evidence that can be regularly received in a forensic lab.

The mixed results observed in the soda can blind study show that the test works on this type of evidence, but due to unknown circumstances, we may not get positive results on every casework soda can tested. Negative soda cans could have had their contents just poured into a cup, or a can could have been washed off prior to disposal.

The specificity study showed that this test has a high specificity for saliva, and did not cross-react with other human body fluids in the casework-like concentrations. Even the dog and cat saliva did not test positive, showing that the test is somewhat specific for human saliva.

In the ski mask casework study, only one of the masks tested positive. On further inspection of the mask that tested negative, we discovered that the cutting we took from came from an area that would not be in contact with the mouth. This shows a case where this test could keep an investigator from sending the wrong piece of evidence for DNA testing.

Conclusion

Through this study, this test has shown to be accurate in identifying saliva present on an item and sensitive to the concentrations of saliva that may be on casework items. The test is also easy to use and the results are easy to read when measured against a negative control, except in cases where the dilution of the saliva approaches the limit of detection. By determining if the yellow color change is strong or weak, the test can also be used in a rudimentary way to see how concentrated or diluted a stain is.

Other studies need to be conducted to further validate this product. Mixture studies should be performed to see if trace amounts of another body fluid can contaminate a saliva sample. A degradation study would also be useful. Saliva stains could be made on filter papers and they could be left outside and inside for varying amounts of time to see if they still give positive results with this test. More people should also be tested to see if different people give stronger, weaker, or even no results compared to others. Other casework trials, such as bite mark swabbings should also be tested.

The success of this test in each part of the study has brought me to the opinion that it is very sensitive, simple, and accurate. Someone can be taught to use it very quickly and it requires no additional equipment or software. By using this product, we can decrease the amount of samples that are sent needlessly for DNA testing when there is no saliva present. With further testing, this product will most likely receive a positive validation and become a useful tool in forensic casework labs.

References

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Figure 1: Extraction Run 1



Figure 2: Extraction Run 2



Figure 3: Extraction Run 3

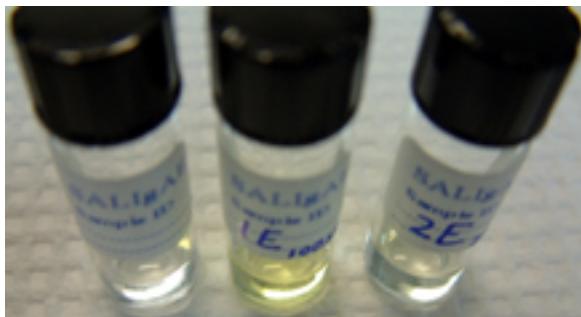


Figure 4: Extraction Run 4



Figure 5: Whole Saliva Dilution Series 1



Figure 6: Whole Saliva Dilution Series 2

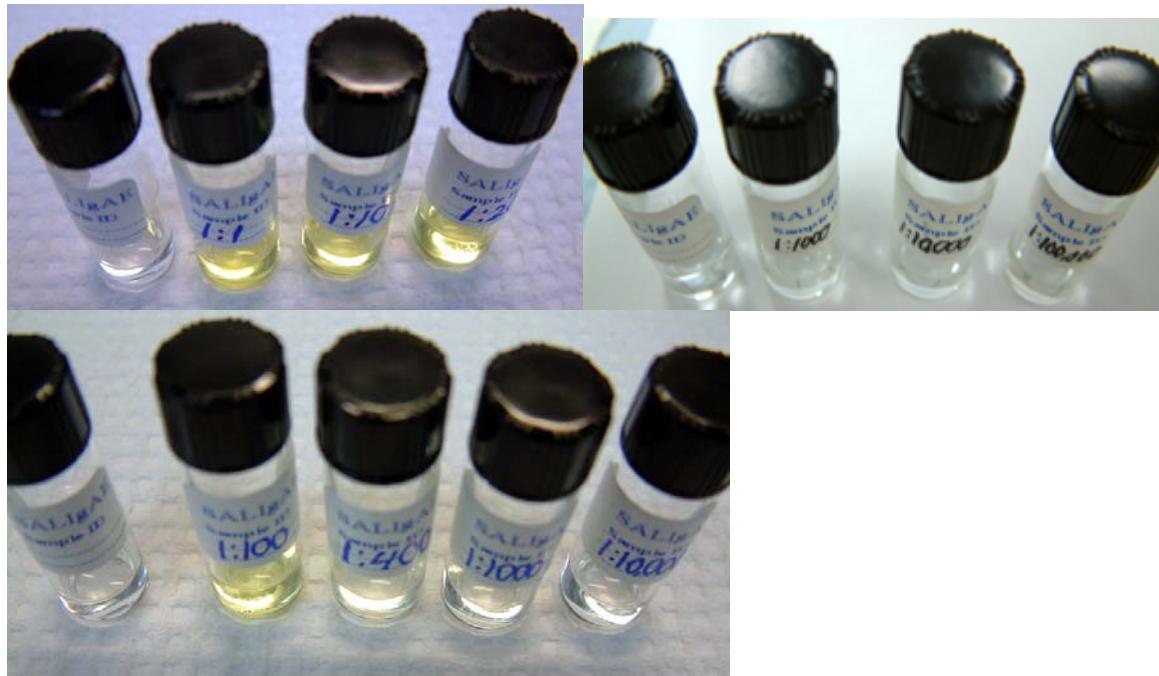


Figure 7: Mock Casework- Envelopes



Figure 8: Mock Casework -Bottle/Can swabbing



Figure 9: Specificity Study



Figure 10: Ski Masks for Ski Mask Casework Trial



Figure 11: Ski Mask Casework Trial



Figure 12: Soda Can Blind Study



Figure 13: Cigarette Filter Blind Study

