# HOW YELLOW IS YELLOW? UV-VIS AND SALIGAE® QUALITATIVE AND SEMI-QUANTITATIVE TOOLS FOR THE ANALYSIS OF SALIVARY AMYLASE

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Human saliva is often encountered in forensic investigations of violent crimes. Detecting the presence of saliva in a biological sample is essential for investigative purposes and for eventual DNA analysis. The SALIgAE® saliva test from *Abacus Diagnostics Inc.* is a simple, sensitive, and accurate test for saliva. It is a colorimetric method and can potentially quantify the concentration of amylase in a sample. Amylase, a constituent of saliva, is found in high concentrations in humans. The color of the SALIgAE® reagent changes from clear to yellow when amylase is present in a sample. However, determining the color change solely by visual means can be subjective, especially if the saliva in the evidence sample is extremely diluted or highly degraded, due to severe environmental conditions.

The goal of this research was to quantify the color change results using ultraviolet-visible spectrometry (UV-VIS). Another aspect of this study was to compare the UV-VIS method's performance with the RSID™-Saliva immunochromatographic test. Both the SALIgAE® & RSID™-Saliva tests require refrigeration, are completed in ten minutes and are currently listed as qualitative assays.

In this study, the UV-VIS method was employed to measure the change in absorbance of a sample at 403 nm in 30 second intervals over a 10 minute period after the addition of the SALIgAE® reagent. Serial dilutions of the standard human salivary amylase obtained from *Meridian Life Science, Inc*. were analyzed by observation of the color change in the SALIgAE® saliva test reagent, by quantifying the absorbance change of the SALIgAE reagent using UV-VIS method and by visual examination of the RSIDTM -Saliva card. Some of the dilutions which indicated positive absorbance with the UV-VIS did not show noticeable color change in the SALIgAE® reagent and tested either negative or weak positive with the RSIDTM-Saliva immunochromatographic card. Therefore, the sensitivity of the SALIgAE® test can be enhanced when color changes are measured by the UV-VIS.

The concentration of salivary amylase dilution just above the lower end of the quantification range for UV-VIS (0.2 to 2) was 1 mg/L or 1 ppm (part per million), which corresponds to 0.518 units of amylase. In addition, the absorbance change and kinetics of the reaction between the amylase and the SALIgAE® reagent are directly proportional to the concentration of the salivary amylase in a dilution. This relationship can be used to quantify the amount of amylase in a sample. Serial dilutions of human saliva samples obtained from several donors were also analyzed, and similar conclusions were drawn from the results. The UV-VIS method may be able to provide an approximation of the quantity of saliva in a sample; therefore, the method should be considered semi-quantitative.

Pancreatic amylase was also examined by the UV-VIS method and by the SALIgAE® test. The results have indicated that pancreatic amylase did react with the SALIgAE® reagent. However, the reaction between the SALIgAE® reagent and the pancreatic amylase was less aggressive than the reaction with the salivary amylase. Other human body fluids including semen, blood, urine, and body fluids from animals such as saliva and urine did not yield positive results with the SALIgAE® UV-VIS method. No color change was detected in the SALIgAE® reagent when using these samples.

This research indicates that the SALIgAE® reagent, in conjunction with the UV-VIS method, can be used to qualitatively and semi-quantitatively measure the amount of saliva in an evidence sample.