B44 Investigating Novel Methods for Estimating Time Since Deposition (TSD) of Bloodstains in Forensic Samples

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The goal of this presentation is to inform attendees of three techniques for estimating the TSD of blood in relation to forensic cases.

This presentation will provide the forensic science community with insight into multiple methods that exhibit potential to estimate the age of bloodstains. These include the measurement of enzymatic activity, protein concentration, and the degradation ratio of two ubiquitously expressed RNAs.

Blood is the most commonly encountered biological fluid found at violent crimes and can provide probative evidence in terms of DNA profiles and pattern analysis; however, these stains can also reveal previously untapped potential in determining the time of deposition of a bloodstain resulting from a trauma or event. The ability to estimate TSD of bloodstains has been researched in the past utilizing many different methods, yet results varied with no complete agreement on one method for implementation into real casework. The goal of this study was to investigate a variety of methods which exhibit potential for TSD estimation. These include investigating over time, enzyme activity, the quantification and spectrophotometric observation of total protein, and the degradation of two RNA species.

Following Institutional Review Board (IRB) approval, venous blood was collected from volunteers with informed consent into sterile EDTA vacutainer tubes. Then 100µL of blood was deposited on white cotton cloth, in triplicate, and allowed to age in a cool, dark environment for 24 hours, 48 hours, 1 week, 2 weeks, 1 month, 3 months, and 6 months. Enzyme activity of Alkaline Phosphatase (ALP) was determined using a colorimetric reaction that was measured using a Nanodrop® OneC Ultraviolet/Visible (UV/Vis) spectrophotometer. Total protein was extracted, quantified, and viewed spectrophotometrically using the UV/Vis spectrophotometer. Total RNA was extracted using the RNeasy Mini Kit, quantified, and expression analysis was performed using Real Time-Polymerase Chain Reaction (RT-PCR) targeting beta-actin and 18 S RNA.

After spectrophotometrically observing the enzymatic activity of ALP, the concentration was determined using a standard curve. It was found that from fresh blood to six-month blood the concentration dropped from 178.9U to 32.36U. When examining the amount of quantified total protein, it was found to have not decreased as drastically, ranging from 5.912mg/mL to 4.981mg/mL in the same six-month period. When each sample is spectrophotometrically observed, three specific peaks are seen at 412nm designated λ, 541nm designated β, and 576nm designated α. These peaks have historically shown correspondence to the derivatives of hemoglobin and decrease in parallel with the conformational changes that correspond to hemoglobin's degradation. The most specific change is found between 541nm and 576nm. These two separate peaks begin to fuse into one smaller peak, relating the eventual conformational change of hemoglobin into hemichrome. The most pronounced peak, found at 412nm, remains present over time; however, it diminishes from an absorbance of 1.52 to 0.50 over the six-month period. Quantifiable amounts of total RNA were extracted from all samples ranging from 22.159ng/µL to 7.000ng/µL, with no real trend of an increase or decrease over time observed. Both Beta-actin and 18S RNA were detected in all samples and have shown a general trend to decrease in expression over time.

This study has shown, using several different methods, the great potential present in the ability to estimate the TSD of bloodstains. Further research is yet to be conducted; however, the results obtained thus far show great promise for the future. It is believed that not one single method will provide the answer; rather, the utilization of multiple methods in concert with each other will ultimately provide the investigator with greater accuracy.

Aging, Blood, Degradation

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