B154 An Exploration of EDTA Detection Within Forensically Relevant Blood Samples

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Learning Overview: After attending this presentation, attendees will be familiar with new presumptive and confirmatory techniques for the detection of disodium Ethylenediaminetetraacetic Acid (EDTA) within blood samples. Challenges encountered during the development of these methods will be discussed, as well as their limitations.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing potential new methods for the presumptive and confirmatory detection of EDTA within blood samples, which can assist in the identification of "planted" blood evidence. Additional insight into possible techniques for the removal of hemoglobin from blood samples will be discussed as a side benefit to forensic biologists.

Detection of EDTA within blood samples has recently resurfaced in the forensic science community, especially as it pertains to "planted" evidence. Any method intended to distinguish authentic blood evidence from planted evidence requires the detection of exogenous component(s), such as EDTA, that would normally be absent in human blood. The ability of EDTA to form a stable complex with calcium, magnesium, and other metal ions enables its use during complexometric titrations to detect and quantify metal ions within a solution.[1] A colorimetric indicator, Eriochrome® Black T, was identified as a possible candidate for use in the detection of EDTA. This indicator changes color when free calcium and magnesium ions are present. If EDTA is present, it will preferentially bind these ions, preventing their interaction with the indicator. As visualization of a color change is inherently subjective and can preclude color-blind individuals from performing this protocol, use of UV/Visible (UV/Vis) spectroscopy could remove such subjectivity. It was hypothesized that the Eriochrome® Black T indicator, in conjunction with UV/Vis spectroscopy, could be used to presumptively detect the presence of EDTA. Furthermore, Attenuated Total Reflectance/Fourier Transform Infrared (ATR/FTIR) and Raman spectroscopy were also explored as possible confirmatory methods for the identification of EDTA. These rotational-vibrational techniques were selected given their high potential for compatibility with EDTA and to supplement previous reports using other instrumentation, primarily chromatographic, for EDTA detection.[2-6]

To establish proof of concept, sensitivity studies were performed based on expected levels of EDTA added to human blood, as well as expected levels of calcium and magnesium normally occurring in human blood, while also factoring in forensically relevant blood sample sizes. EDTA standards (0.003–3mg) were utilized for all four methods, whereas calcium and magnesium standards (1–30µg) were also necessary for the colorimetric indicator test and subsequent UV/Vis. For all EDTA amounts tested, Eriochrome® Black T correctly indicated whether EDTA was present or not, in combination with ≥1µg of calcium and ≥0.1µg of magnesium, as long as the EDTA:ion ratio was ≥3 for calcium and ≥10 for magnesium. Normally occurring EDTA to calcium or magnesium ratios well exceed these values. Furthermore, resulting UV/Vis spectra were different between samples that tested positive and negative for EDTA using Eriochrome® Black T. Positive samples (blue) yielded a peak around 200nm; all other regions of these spectra exhibited negligible absorbance. Negative samples (pink) exhibited a weak, broad peak from 500nm–700nm, with a point of inflection around 590nm. Unfortunately, robust baselines could not be established at any of these regions, thereby preventing the formation of reliable conclusions. Last, testing with ATR/FTIR and Raman spectroscopy proved challenging with the small sample sizes tested and need additional troubleshooting.

Following proof of concept, various areas were explored to adapt the Eriochrome® Black T indicator for use with forensically relevant blood samples. Sample size was a significant concern and quickly led to the need to remedy color interference from blood itself, even from dilutions as low as 1:400. Several products or reagents were evaluated: Microcon® filters, dryer sheets, HemogloBind™, sodium hydroxide, ethanol, and amidine latex beads. Though many of them proved successful at removing the interfering red color from blood, nearly all adversely interfered with the indicator reaction and resulted in either false positives or negatives. Unexpectedly, performing a 96% ethanol wash was effective and yielded the expected results; this process will be pursued further. Transitioning from liquid blood to dried stains was also problematic anytime a cellulose-based material was used (specifically, cotton swabs and cotton T-shirts); use of foam swabs remedied this issue.

These methods show varying degrees of promise for the development of a robust EDTA detection process. Future work will aim to further optimize and validate suitable methods.

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EDTA, Blood, Planted Evidence

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